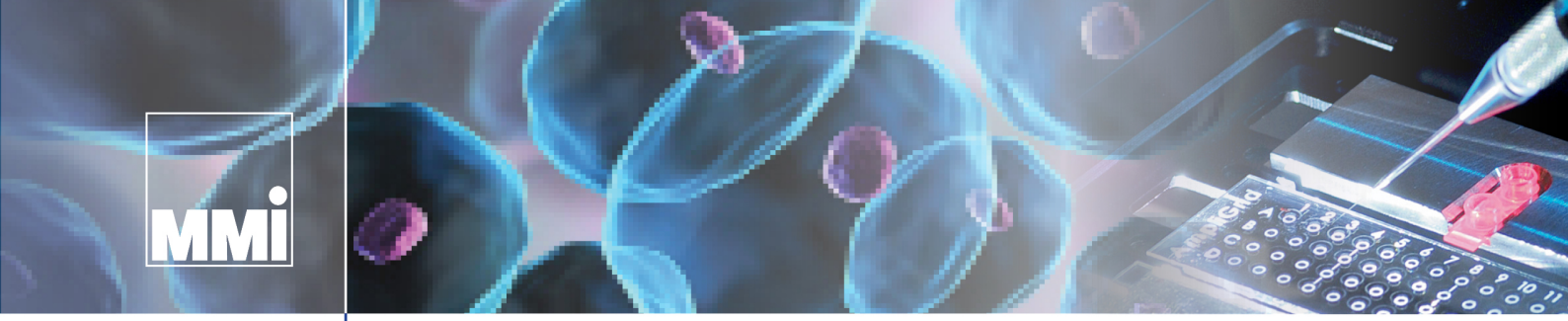

mmi CellEctor

User Manual

Molecular Machines & Industries AG
www.molecular-machines.com

User Manual: MMI CellEctor
Version 4.3
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MMI AG
Flughofstrasse 37
8152 Glattbrugg
Switzerland

Technical features are subject to change without notice

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1 Security advice

1.1 General safety

- Do NOT disassemble the system. The installation of the system is done by MMI service personnel or a designated MMI representative. Repairs, removal or exchange of components except for the operations described in this manual must be carried out solely by the MMI service personnel or persons expressly authorized by MMI. If you have any problems with the instrument, contact MMI.
- The responsibility for the correct functioning of the mmi CellEctor passes onto the owner or user if the system is maintained or serviced improperly by persons not belonging to the MMI service team or if used for a purpose other than that intended. MMI is not liable for damage occurring from non-observance of these notes.
- The power supply is installed by MMI. MMI ensures that the system is provided with the appropriate voltage. Do not change the power cords.
- Avoid wet or dusty conditions near the system. Do not attempt to use the system if liquids get inside it. Contact MMI in such a case.
- Unplug all the electrical supply before cleaning the system. Do not use cleaning fluids or sprays; use solely a smooth and dry cloth.
- If the stage is not calibrated, its movements can be sudden and fast. Ensure that the work area around the stage is free of clutter and material.
- Read the manual of your microscope for microscope specific precautions. If you do not have the manual, contact your microscope provider or MMI.

1.2 *mmi CellEctor* safety

- *mmi CellEctor* uses very thin glass capillaries that may break into splinters and injure eyes or other parts of the human body. Thus, it is compulsory for the user to wear protective gloves and clothes while working with the *mmi CellEctor*.

- Never point capillaries at persons. Capillaries can suddenly be loosened from their grips under high pressure and become missiles.
- The *mmi CellEctor* is to be used solely for collecting cells in accordance with the appropriate legislative requirements.
- Please ensure that all work is carried out in accordance with this manual.

2 Installation

The MMI system may only be installed by an MMI service engineer or our designated representative in the laboratory of the customer. After the installation training will be provided in the use and operation of the system. The customer should not change the installation of the equipment.

Should you want to move an installed unit, please contact MMI for assistance. With any malfunction of the device, please contact our service department:

service@molecular–machines.com

After installation or modification of the MMI system, an authorized specialist must perform a thorough check to ensure that the system is in perfect condition. If your system comprises a laser, it must be ensured that the laser safety features are functioning correctly and that the covers to protect against laser radiation are fitted.

3 The mmi CellTools instrumentation family

The mmi CellTools are a fully modular instrumentation family, including the following components:

- *mmi CellCut*: laser microdissection to isolate single cells or areas of tissue
- *mmi CellManipulator*: optical tweezers to manipulate cells or beads with an optical trap
- *mmi CellEctor*: automated micro-pipetting to mechanically manipulate cells or beads with a capillary and mechanical micromanipulator
- *mmi CellExplorer*: pattern recognition software for PC based image analysis

Any or all of these modules can be combined in one microscopic environment.

4 Getting started

4.1 System start-up

To start the mmi CellTools® with mmi CellEctor® follow the steps below:

- Start up the PC and allow the boot process to complete, reaching the Windows desktop.
- Turn on the microscope control box.
- Start *mmi CellTool* software and wait until the software has finished the start up and self-test procedure. For the system to work properly, all the hardware components have to pass the hardware monitoring procedure without errors.
- Remote control of the microscope is compulsory for the *mmi CellEctor* to achieve the highest precision in the capillary vertical positioning with respect to the slide surface. If the remote control is off, or you work with a manual microscope, only a rough positioning of the capillary is possible.

4.2 System preparation

Follow these steps:

- Ensure that the following microscope settings are correct:
  - camera orientation
  - objectives calibration
  - illumination settings for each objective
  - camera settings for each objective

  On new systems, this has already been done for you by the service technician.
- Prepare the mmi CellCellector® for work as described in sections 6.8 and 6.9

4.3 System turn off

- Shut down *CellTools* by using the

  Project → *Exit*

  command or press the close button in the top right corner of the program window.

- Switch the microscope off.

- Shut down the PC by using the standard Windows® command.

- Let the illumination housing of the microscope cool down to the room temperature and protect the system with the microscope cover.

5 mmi CellTools – Main application

5.1 Main window and plug-ins

Fig. 5.1 shows the extended tool area and explains the main components of the *mmi CellTools* user interface.

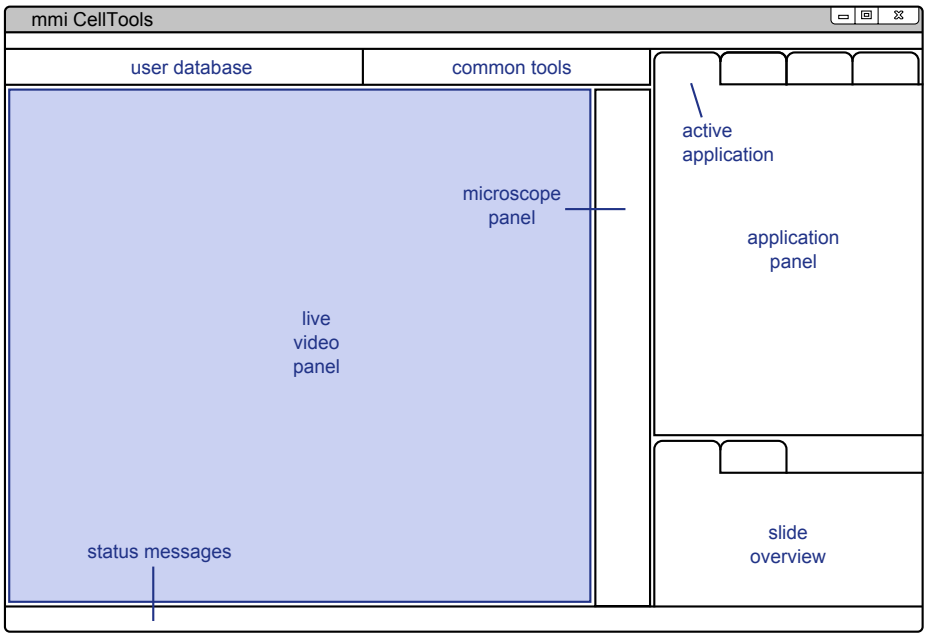


Figure 5.1: *Structure of the mmi CellTools user interface*

The major portion of the window is occupied by the **live video panel**, which displays the current field of view of the microscope and serves as the main area for interaction between the user and the system.

mmi CellTools is a single piece of software that controls all micromanipulation devices of the mmi instrumentation family (see chapter 3). The instrument-specific controls are located in the **application panel**. Switch between applications using the tabs at the top.

Those user interface controls that are common to all applications are located in the tool bar at the top of the window. The **user database** controls provide access to instrument parameters for specific samples, for certain imaging situations, and for each objective (see section 5.2). The remaining **common tools** on the tool bar provide elements for camera and stage control.

On systems equipped with an automated microscope, the microscope panel provides access to z-drive (focus) control and other microscope-specific features (see chapter 7). The **slide overview** panel provides controls for slide scanning and navigation (see section 5.5.2). Finally, the **status messages** bar at the bottom displays current stage coordinates and camera frame rate.

To accommodate for left-handed users, the application and microscope panels can be moved to the left-hand side using the menu item

Edit $\rightarrow$*Left-handed UI*

5.2 User-specific database

All settings saved in the *mmi CellTools* are unique to the current user logged in. *mmi CellTools* fully supports Microsoft Windows user management. During program startup the last settings saved by the active user are loaded.

The database represents a hierarchical structure:

Slide	$\Longrightarrow$	Setup 1	$\Longrightarrow$	Objective 1
			$\Longrightarrow$	Objective 2
			$\vdots$	
	$\Longrightarrow$	Setup 2	$\Longrightarrow$	Objective 1
			$\Longrightarrow$	Objective 2
	$\vdots$		$\vdots$	

Basically the user can save all parameters for each experiment (Slide) he/she runs separately. If the user changes settings for an experiment, settings from recent experiments are still saved and accessible.

The Setup represents all necessary parameters to define an illumination method (bright field, fluorescence, DIC. . .). If you change a parameter in a setup, the change will only be reflected in the current slide.

The Objective represents all objective related settings and calibrations. If you change an objective calibration or objective related parameter, the change only will be reflected in the current setup and slide.

5.2.1 Slide editor

The slide selection box contains all samples you defined in the past. All documentation is saved under this name. To change the database open the slide editor (Fig. 5.2) by pressing the edit button.

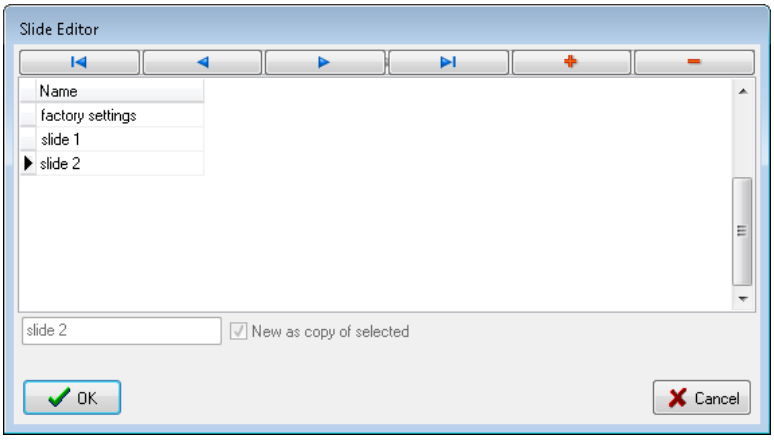


Figure 5.2: *Slide editor*

Use the + button to define a new slide. All parameters from the active slide will be copied. Rename the slide by clicking on the name. Use the − button to remove the active slide.

5.2.2 Setup editor

The setup selection box contains all parameters defined for the active slide. To add, remove or change the database press the edit button. To use this editor follow the same steps as for the slide editor described above. If you create a new slide all setups will automatically be copied from the active slide.

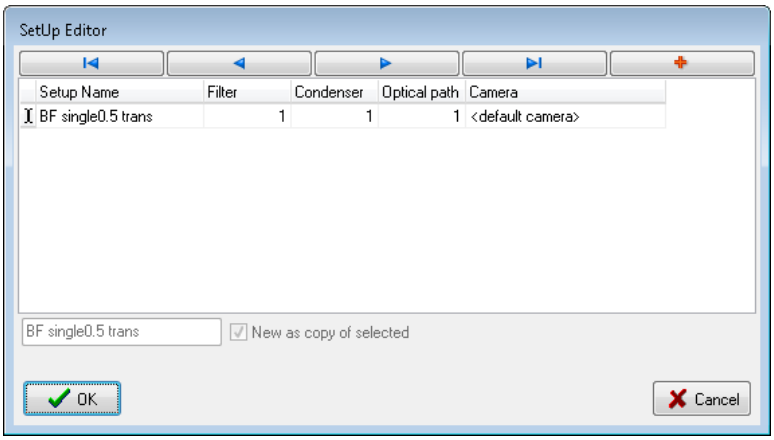


Figure 5.3: *Setup editor*

If you run different types of experiments, e.g., microdissection with bright field, microdissection with fluorescence, or optical tweezers it is recommended to define one setup for each of these experiments.

To rename the defined setups click on the setup name and type in a different name.

The **Filterblock**, **Condenser** and **Opticalpath** settings are only used with motorized microscopes. The use of these parameters is explained in section 7.2.

If your system is equipped with more than one camera, different setups can be associated with a certain camera. You can then switch to a specific camera by selecting a setup defined for that camera.

The camera associated with a setup is displayed in the setup editor (column **Camera**) and can be changed by clicking on the name. The camera used should also be reflected in the setup name. On single-camera systems you should normally select “<default camera>”.

5.2.3 Objective editor

The objective selection box contains all objectives defined for the active setup. To change the database, press the edit button to open the objective editor (Fig. 5.4). To use this editor follow the steps for the Slide Editor described above. If you create a new setup, all objectives will automatically be copied from the active slide.

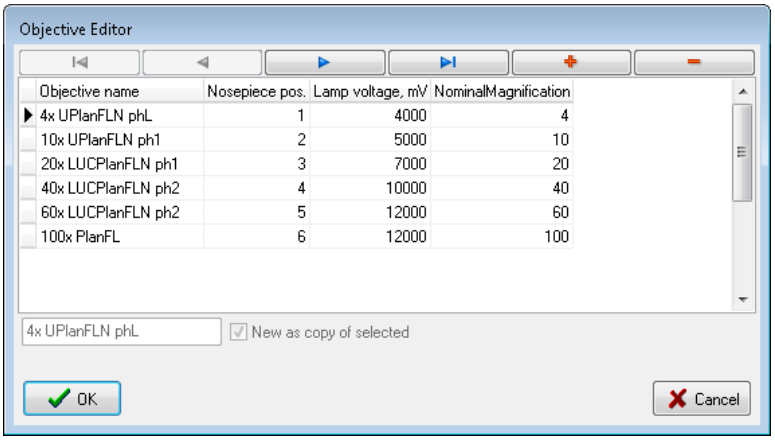


Figure 5.4: *Objective editor*

To rename the defined objective click on the **objective name** and type in a different name.

The **Nosepiece** and **Lamp Voltage** settings are only used with motorized microscopes.

If specified, **Nominal magnification** will be used to compute various objective-

dependent properties.

In addition to the parameters displayed in the Objective Editor window, the following information is stored separately for each objective:

- Camera settings (see section 5.3.2)
- Objective related stage calibration (see section 6.9.1)
- Lens offset (see section 6.9.3)

If one of these settings is not correct, please refer to the corresponding chapter.

5.2.4 Default database reconstruction

Each user handles his/her own database. Changes in this user-specific database will not be visible for any other user.

A default database is always stored separately in a central file location. This default database is specific for your instrument and will be set up and handled only during installation and service.

If for some reason, a user's database becomes unusable, the user can recover the default database by the following procedure:

Procedure

1. Close *mmi CellTools*
2. Delete the following folder from the hard disc:

   `C:\Users\`*username*`\AppData\Roaming\MMImCUTDataBase`

   or for Windows XP Systems:

   `C:\Documents and Settings\`*username*`\Application Data\MMImCUTDataBase`
3. Open *mmi CellTools*

The default database will automatically be recovered and is directly visible in *mmi CellTools*.

You will lose all slide and setup data contained in the user database. You will need to recalibrate the entire system.

5.3 Camera operation

mmi CellTools supports a range of scientific digital cameras, supplied by MMI or third parties:

- The mmi CellCamera range of digital cameras for general microscopy applications
- The Andor iXon^{EM} EMCCD camera for ultra-sensitive detection

5.3.1 Multiple cameras

mmi CellTools also supports multiple cameras. If more than one camera is connected to the system, switch between the cameras by selecting a setup dedicated to that camera.

To define the camera used by a setup, open the setup editor and click on the camera field showing `<default camera>` as default. A drop down list shows up in which you simply select the camera you like to connect to that setup.

> Do not use the setup editor to switch between cameras. The setup editor is only used once to create (at least) one setup for each camera. Then, change cameras by selecting the corresponding setup.

5.3.2 Camera settings

For best imaging results the camera can be controlled through a settings window (Fig. 5.5). To open it, click the camera button in the toolbar or select

Video → *Camera settings*

from the menu (*Ctrl + R*).

Image exposure is controlled through the top elements in the dialog. Unless **Automatic exposure** is selected, the exposure time can be controlled through the **Exposure time** slider or the adjacent input field. In **Automatic exposure** mode the slider is inaccessible. If the auto-exposed images are too bright or too dark, use the **Exposure correction** slider to compensate.

Finally, the amplifier **Gain** can be adjusted to yield brighter, but noisier images. Higher gain settings will, however, reduce the required exposure time. For high quality images (e.g. for publications) it is preferable to use a longer exposure

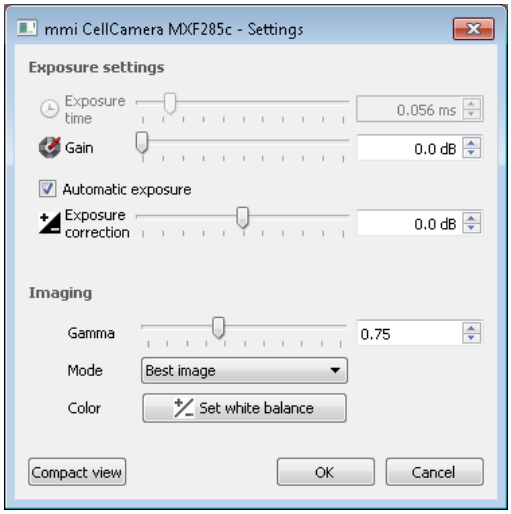


Figure 5.5: *Camera settings for mmi CellCamera models*

time and low gain. Note that the allowed gain range is greater when automatic exposure is off.

The controls in the bottom part of the dialog allow control over color and contrast. Use the **Gamma** slider to control image contrast. The default Gamma value is 0.75, and it may be adjusted over a range of 0.01 to 2.0. Low gamma values are recommended especially to brighten low light fluorescence images. High gamma values reduce noise and improve the black level of the image.

The camera **Mode** provides color settings optimized for various applications. For bright field, the color quality can be optimized with the **Best image** mode. The camera transfers a high quality YUV4:4:4 data stream with full pixel resolution. By selecting **Fastest rate**, the image rate (frames per second, fps) will be maximized. In this mode up to 20 fps (depending on your camera model) with full pixel resolution are displayed. For fluorescence applications, the contrast can be optimized with the **Low light** option. The camera transfers a high quality RGB data stream with full pixel resolution. This setting is used mainly in combination with the **Gamma** and **Gain** setting. If you select **Binning**, the camera operates at a lower resolution, but yields brighter black and white images at a very fast frame rate.

Color shifts, mainly caused by changes in lamp brightness, can be corrected using the white balance function. To set the white balance, first locate an empty, transparent part on the sample slide, then click the **Set white balance** button. If the image is too bright or too dark the white balance fails.

The white balance function is also available in the drop down menu next to the camera button, in the main menu under

Video →Adjust white balance

or using the keyboard shortcut *Ctrl + W*.

Do not use the Set white balance option in fluorescence imaging.

All camera settings, including white balance, are stored separately for each objective. This eliminates the need to adjust the camera after each objective change. Optionally, the software uses a single white balance setting for all objectives. This is recommended for non-automated microscopes. Select

Video →Save white balance per objective

to enable or disable this function.

5.3.3 Freeze video / live video

In fluorescence applications it makes sense to freeze the video when you have acquired a good image. After freezing the video you can close the fluorescence shutter and go ahead with drawing and cutting your dissectates without further photo bleaching of the fluorescence dye.

To freeze the video use the menu item

Video →Video freeze

To go back to live video presentation select

Video →Video live

5.3.4 Saving images

To save an image simply press the **Save image** button, use the menu item

Video →Save Image

or press *Ctrl + S*.

The file dialog allows you to specify the image filename and image type (JPG, BMP, PNG and TIF). The image will be saved with maximum pixel resolution. By selecting the **Include drawings** check box you can save a screen shot from the current video image including all markers and drawings.

When saving images with drawings, the system will produce a screenshot from the live video area, resulting in reduced pixel resolution of the saved image.

Press the **Copy image to clipboard** button to make the current image available to other applications. The image will be copied to the Windows clipboard in order to paste it into e.g. office and image processing applications. The same result can be seen with the menu item

Edit →Copy Image

The **Include drawings** does not affect the **Copy image to clipboard** function. The clipboard always receives the original camera image.

5.3.5 Recording movies

mmi CellTools allows you to record live camera images into video files (AVI format). It supports compressed and lossless video codecs and allows you to record time-lapse movies.

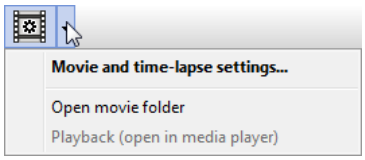


Figure 5.6: *Movie recording functions in the tool bar*

Click the **Record** button to start recording a new movie. CellTools will automatically create a new AVI file in the movie folder and record video until you press the **Stop** button. If **Auto-open** is enabled (see below), the movie will open in Media Player immediately after recording. Otherwise, you can access recorded movies through the drop-down menu items *Playback (open in media player)* and *Open movie folder*.

By default, the movie folder is located in

`My Documents\mmiCellTools\Movies`.

Movies are compressed with a video codec, currently either Windows Media Video 9 or XVID. To play back those movies, your computer will require installation of the same codec. Codecs are found on the mmi CellTools installation media.

Also note that any shapes drawn will not be recorded.

5.3.5.1 Movie settings

Detailed aspects of movie recording can be controlled by opening the **Movie settings** dialog (Fig. 5.7).

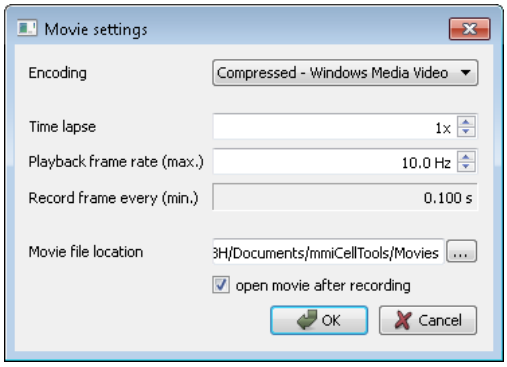


Figure 5.7: *Movie settings*

The **Encoding** specifies the format (codec) to store the video frames. Table 5.1 gives an overview of available encodings and their application.

Table 5.1: *Movie encoding options*

Encoding method	Image quality	Recommended for
Uncompressed	Lossless	Short videos Extracting frames in full quality
Compressed – Windows Media Video (default)	Lossy	General use Playback primarily on Windows systems.[1]
Compressed – XVID	Lossy	Color video (not usable for black and white cameras) Playback on any system with XVID codec installed

To create basic time lapse movies only adjust the **Time lapse** factor as desired. This factor defines the speed at which the movie will play back. Enter “1x” to turn off time lapse (standard time).

The **Playback rate** specifies at how many frames per second the final video should be played back. (This is identical to the recording rate unless using time lapse.) The default playback rate is 10 Hz and should be suitable for general use.

The **Recording interval** specifies the minimum time interval between two recorded video frames. The recording interval is computed from time lapse factor and playback rate and is for information only.

[1] To play back Windows Media Video files on Mac OS X, install Windows Media Player for Mac OS (Flip4Mac), which is downloadable from the Microsoft website.

Depending on the current camera settings and available computer hardware it may not be possible to achieve very short recording intervals.

To control the rates at which frames are recorded and played back, change the **Playback frame rate**. If time lapse is off, the movie is played back at the same rate at which it was recorded. When using time lapse, the recording rate is automatically adjusted. The dialog displays the recording interval, i.e. the time elapsed between two frames. This interval significantly influences the size of the resulting video file.

The **Movie folder** input field allows you to specify the destination folder where movie files are saved. For maximum performance, this should not be a network folder. Check the **Open movie after recording** option to have movies automatically open and playback in Media Player after recording.

5.4 Motorized stage control

The movement of the motorized stage is controlled by the *mmi CellTools* software.

5.4.1 Mouse movement

Choose the move mode by clicking the hand tool button.

In the move mode the cursor in the video panel always appears as a hand. By clicking and dragging the left mouse button, the stage directly follows the mouse movement.

If stage movement does not exactly follow mouse movement, you may need to carry out an objective calibration (see section 6.9.1).

You can quickly switch to move mode and back again by pressing *Space*.

5.4.2 Keyboard movement

The main arrow keys and numeric pad arrow keys can be used to move the stage in discrete steps or continuously at constant velocity (Fig. 5.8).

A single press of a cursor key moves the stage by a defined distance. By default, this is 10% of the screen for the cursor keys and 90% of the screen for numeric pad keys.

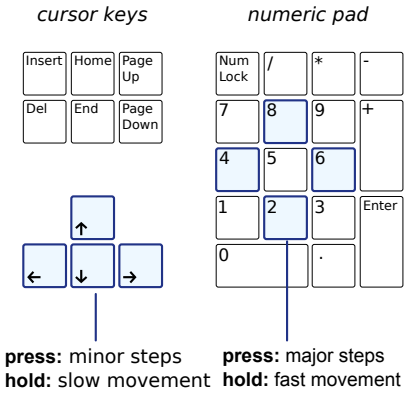


Figure 5.8: *Stage movement using the keyboard*

NumLock must be turned on for numeric cursor keys to work as expected.

For moving larger distances, hold down the respective key. The velocities for the two sets of movement keys can be set independently (see section 5.4.3). By default, the cursor keys move slowly and the numeric pad keys move fast.

In applications where you need movement by well-defined distances only, the continuous movement can be suppressed by enabling *Caps Lock*.

5.4.3 Stage movement settings

To change stage movement settings, press the **Stage movement settings** button, click the menu bar item

Stage →*Stage movement settings*

or press *Ctrl + P* to get the window in Fig. 5.9.

Acceleration This value determines the stage motors' acceleration for both keyboard and mouse movement.

Reducing this value may facilitate the handling of liquid suspension samples. For all other samples we recommend using 100%.

Units For the arrow keys on the keyboard you can set the stage settings in two different units:

- Screens (or percentage of screen)
- Micrometers (µm)

When using micrometer units, note that you may have to adapt distances with every objective change.

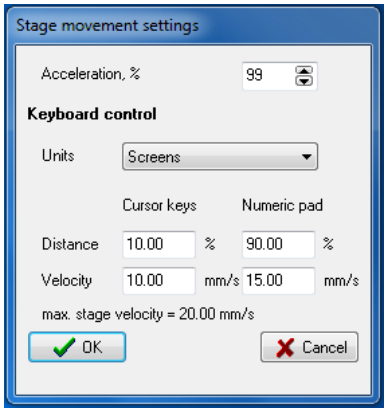


Figure 5.9: *Stage movement settings*

Distance and velocity These values can be chosen independently for cursor and numeric pad keys. By default, cursor keys are used for minor steps and numeric pad keys for major steps.

Values that are outside the allowable range will be shown in red.

5.5 Slide navigation and scanning

5.5.1 Mechanical limit calibration

In order to use the navigation and scanning features, *mmi CellTools* needs to know the the position and dimensions of the sample. This is the purpose of the mechanical limit calibration.

Under normal circumstances, the calibration remains valid unless the stage is moved manually or using a software other than *mmi CellTools*.

If the software detects an invalid calibration, slide scanning will be disabled and the overview scan will show a red warning sign.

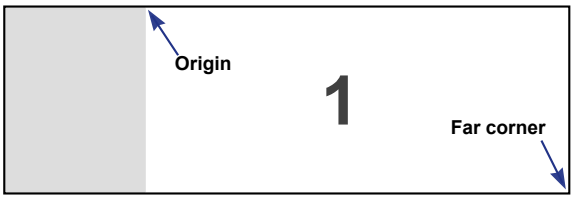
Procedure

1. Use a small magnification objective (e.g. 4x) and move the stage to slide 1 (rearmost slide).
2. Move the stage such that the top-left corner is just visible at the edge of the field of view.
3. Select *Define origin* from the **slide geometry** popup menu (bottom right corner of the overview panel).
4. Move the stage such that the bottom-right corner is just visible at the edge of the field of view.

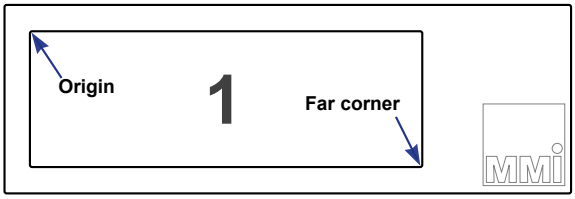
5. Select *Define far corner*.

You are now ready to use slide navigation and scanning. The field-of-view indicator and the dimensions of the slide overview will adapt during calibration.

The suggested positions to use as limits for different types of slides are illustrated in Fig. 5.10.



(a) Glass slide



(b) mmi Membrane slide

Figure 5.10: *Suggested mechanical limit positions on standard microscopic slides*

The mechanical limit calibraiton needs only be carried out on the first slide. The other slides automatically use the same mechanical limits.

5.5.2 Overview scan

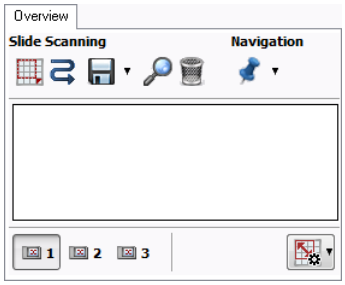


Figure 5.11: *Slide overview*

The overview scan is started by pressing the **Start scan** button. This window shows you an overview over your sample (the “roadmap image”). If no area of interest is defined, the whole slide will be scanned within the limits set in chapter 5.5.1.

The current field of view is indicated with a red blinking rectangle or point. You can move this red frame by clicking and dragging the left mouse button.

The motorized xy-stage moves automatically to the chosen detail. With this navigation method you always see the position on the slide.

You can also move to the position of interest by double clicking into the overview area with the left mouse button.

Define the area of the scan with the **Select area** tool. After pressing the button you can select the area of interest in the overview window using the PC mouse. Only the area of interest will be scanned.

Begin the scan by pressing the **Start Scan** button. You can always interrupt the scan with the **Stop scan** button. Also pressing the *Esc* key will interrupt the scan.

The scanned image can be made available to other applications. Use the **Save** button or the corresponding menu item to save the overview image to disk. Alternatively select *Copy image to clipboard* from the popup menu next to the **Save** button to copy the overview image into the Windows clipboard and paste it in other applications.

To erase the scanned image, click on the **Clear overview image** button.

With the **magnifier** button you can open the large slide-overview window (Fig. 5.12).

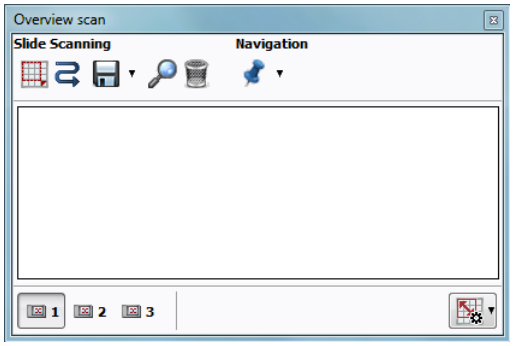


Figure 5.12: *Large slide-overview window*

The window provides the same tools as the small slide-overview panel.

Only the magnifier button behaves differently. In this big slide-overview window the magnifier button opens the standard Windows photo viewer.

5.5.3 Multi slide handling

The *mmi Multislide* holder grants the user the opportunity to use more than one slide in the xy-stage.

To move from one slide to another you can directly select the **target slide** button.

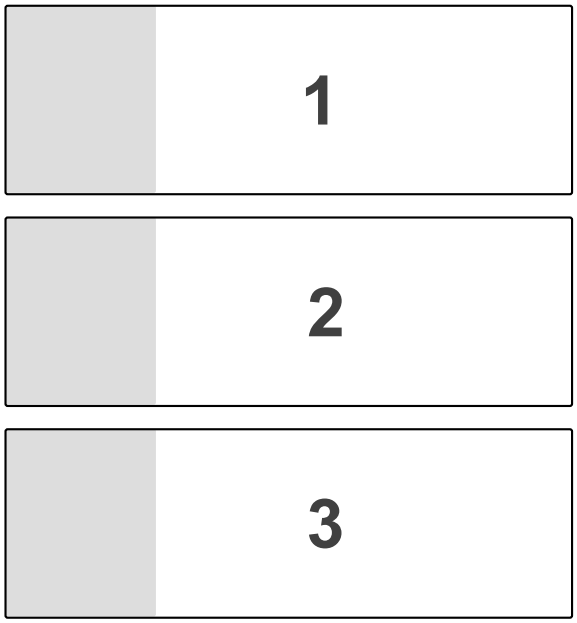


Figure 5.13: *1x3 Multi Slide*

Alternatively you can move to another slide with the keyboard arrow keys. The slide number indicator will automatically adjust to the current slide.

If the number in the slide number indicator does not fit with the position of the slide holder, move the stage to slide position 1 with the keyboard arrow keys and redefine the slide origin as described in chapter 5.5.1.

5.5.4 Pin positions

With the **pin** button you can save the current stage position. By clicking on the arrow to the right you obtain the pin positions menu (Fig. 5.14).

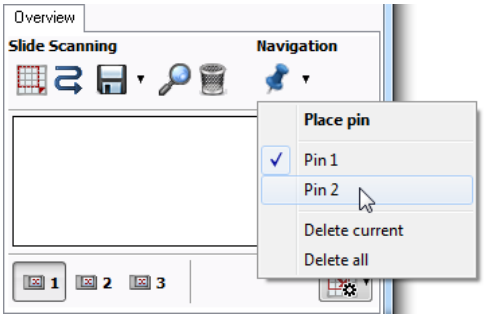


Figure 5.14: *Pin positions*

You can select a pin, which moves the stage to the respective position. The last two entries enable you to delete either the currently selected or all pin positions.

5.6 Distance measurement

Select the measurement tool to measure distances on the sample.

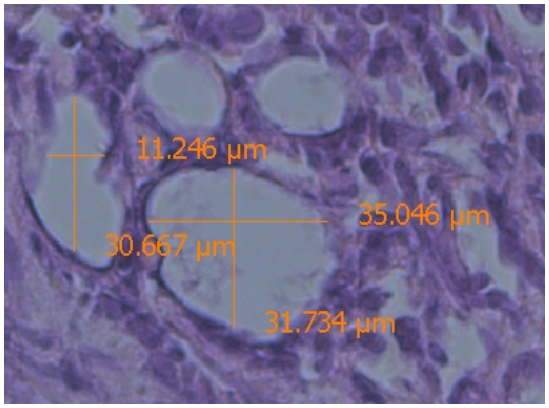


Figure 5.15: *Distance measurement*

Press the left mouse button and drag the mouse. After releasing the mouse button you can see the measured distance (Fig. 5.15)

5.7 Calibration

5.7.1 Camera orientation

To check the camera orientation:

- Using the 40x objective, move one small and obvious point of the sample to the upper-left corner of the video panel.
- Now press the right arrow key in the number pad. (Best using standard step sizes for big step movement: 90% of screen)

If you move the object horizontally to the right upper corner and the object is still on the same level, the camera orientation is correct. If the result shows a shift between the two corners (ie. the object does not move perfectly horizontally), loosen the hex screws on the camera port and rotate the camera carefully. Repeat this “move and observe” operation again until the object moves on the same level across the monitor view. When the object moves in a straight line, the camera orientation is correct. Retighten screws carefully.

5.8 Multi-user report

Users can get information about time spent using *mmi CellTools* for each Windows user account.

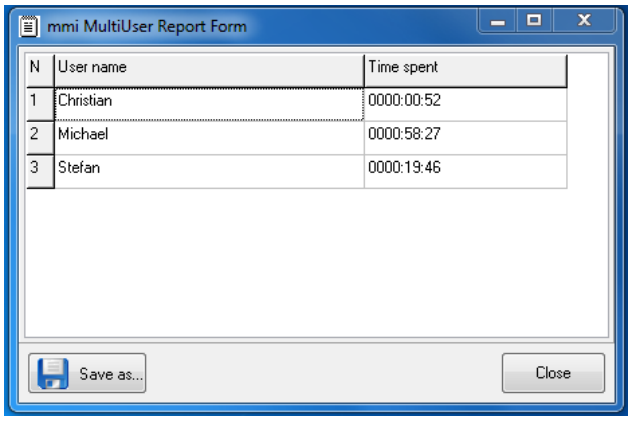


Figure 5.16: *Multi-user report*

Select the menu item

Project →Usage report

or start it from

Start →All Programs →mmi CellTools →mmi MultiUser Report

A time format is hhhh:mm:ss.

5.9 Help

5.9.1 Help topics

The user manual can be opened directly inside the *mmi CellTools* software. By pressing *F1* on the keyboard the PDF file of the User Manual shows up. You also launch the user manual by selecting the respective item in the Help menu.

5.9.2 MMI online

If your PC is connected to the internet you can launch the MMI web page by the menu item

Help →MMI online

Questions about the system can be sent to MMI service staff by the item

Help →Online Support

5.9.3 Version info

Information about the currently installed software version can be found under

Help →Version info

6 mmi CellEctor

6.1 Principles

The molecular analysis of pure enriched cell populations or even of single cells is an important prerequisite of medical genomics and proteomics and may become essential for molecular patient profiling in the future. Up to now this goal is often very difficult and tedious to accomplish. Therefore, MMI developed a new analysis and cell sorting device: the mmi CellEctor. The isolation of single and rare cells from tiny sample sources is a prerequisite for their genotypic and phaenotypic characterization. The mmi CellEctor is the starting point for a new integrated work flow. It facilitates the automated isolation of any type of single and rare cells in three steps:

- Cell recognition
- Cell acquisition
- Cell deposition

The *mmi CellEctor* is based on:

- automated microcapillary
- automated pump
- motorized stage

To encrease the automation level the *mmi CellEctor* can be upgraded with:

- motorized microscope
- *mmi CellExplorer*, the MMI cell recognition solution

Acquisition and deposition are controlled by a high precision pump. This allows establishing protocols that use nanoliter volumes for the separation process and provides the basis for molecular downstream analysis in only 1µl.

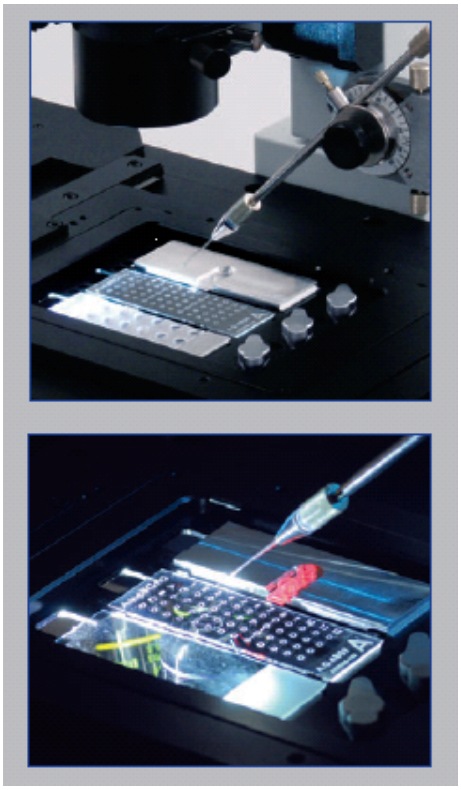


Figure 6.1: *Sample area of the microscope with a ampligrid and microcapillary.*

There is no mechanical stress to (non-adherent) cells: the acquisition occurs solely by flow of the liquid surrounding a cell. Under optimum conditions, even partly adherent cells can be acquired in this way.

There is no contact between the cells and the capillary. The capillary diameter (10, 15, 20, 30 or 40 µm) may significantly exceed that of the cell. For example, cells of 6 µmdiameter can efficiently be acquired with a capillary of 40 µm diameter.

The suspension of cells can be placed either on a flat standard slide or in a dedicated container, e.g. an IBIDI µ-Slide.

The deposition of cells may occur on various targets (deposits). Grid deposits are matrices of either spots (like AmpliGrid) or small containers (like IBIDI well slides). Single spot deposits may consist of a transparent cap, a PCR tube, or a microfluidic device. Whatever the kind of the target is, its size must not exceed the standard slide size that can be inserted in the multi-slide holder of the stage.

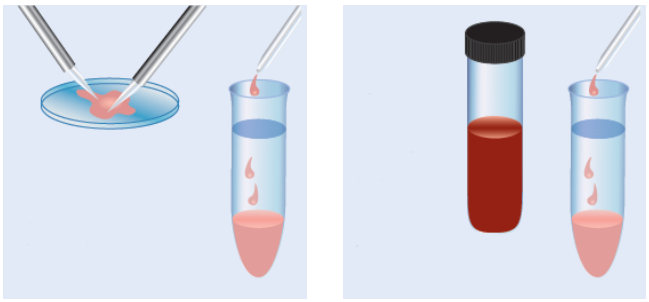
The acquisition and deposition of cells can proceed in a variety of modes: from manual mode through full automation with cells recognition by the mmi CellExplorer. However, even in the manual mode, there is no need to manually operate the hardware components of the system (pump, capillary): all the operations are launched at PC by the user.

The mmi CellEctor is an adjustable, open platform for any assay format. It closes the gap between frequent and rare cell sorting. It is dedicated for the use in the following disciplines:

- cancer research & oncology
- immunology & virology
- stem cell sciences

as well as many other applications in biomedical research, cellular diagnostics, microbiology & virology, environmental ecology, forensics. MMI is committed to establish specified and detailed application-related protocols in a close cooperation with the users.

All the methods applied to measure molecular changes require 100% pure cell populations to ensure unbiased results. For the analysis of DNA, copy number variations or single nucleotide polymorphisms are being measured. Changes of RNA expression are analyzed by microarrays or massive parallel sequencing approaches. Protein patterns are analyzed with spectroscopic or biochemical protocols, and epigenetic changes are detected by changes in methylation patterns. FACS-sorting and bead-based protocols are the major state-of-the-art methods to isolate single cells. Also a wide variety of physical and immunological methods are employed. The *mmi CellEctor* helps to overcome the hurdles and drawbacks of the above mentioned methods. The *mmi CellEctor* technology has been used for the enrichment of tumor cells and immune cells from disaggregated lymphnode material, blood and bone marrow of cancer patients. For the outlined workflow of an application example, the following purification steps were established: Lymphnode metastasis is dis-



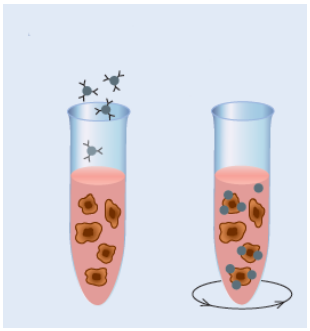
(a) Preparation of cell suspension: disaggregate lymphnodes and filter cells

(b) Lyse erythrocytes from blood or bone marrow samples

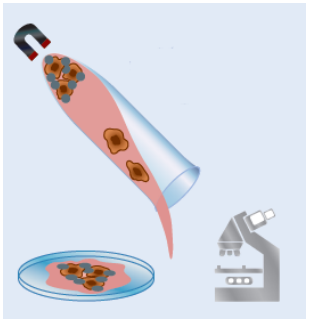
Figure 6.2: *Preparation of the patient material*

aggregated by the use of scalpels and filtered through a 70 micron filter to get a cell suspension. Blood and bone marrow is treated by erythrocyte lysis, to remove present erythrocytes, Tveito et. al. 2007 [1]. In a first enrichment step,

[1] Reference: Tveito, S., Maelandsmo, M. G., Hoifodt, K. H., Rasmussen, H., Fodstad, O.:"Specific isolation of disseminated cancer cells: a new method permitting sensitive detec-

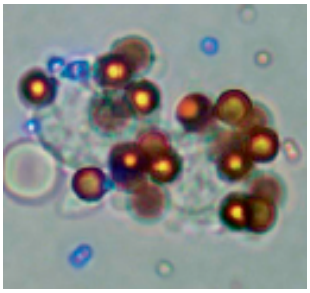


(a) specific immunomagnetic beads and incubate under rotation

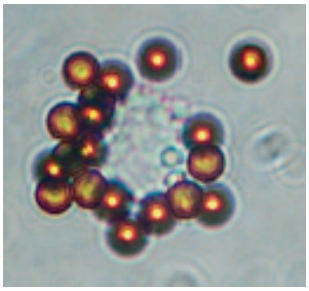


(b) magnetic separation of immunomagnetically rosetted cells, microscopic evaluation and *mmi CellEctor* mediated cell picking

Figure 6.3: *Pre-enrichment of target cells by immunmagnetic selection via targeting a cell specific surface marker and mmi CellEctor mediated cell picking.*



(a)



(b)

Figure 6.4: *Lymphnode metastatic cells targeted with an anti EP-CAM antibody*

paramagnetic beads coated with an appropriate surface-antibody were used for the immunomagnetic enrichment. In the second step the mmi CellEctor technology is used for individual cell picking.

6.2 Slide Types

The slides carrying the suspension of cells (sample) or serving as target for cells have to fit the standard slide size of approx. 76 x 26 mm. Within this constraint, a variety of slides can be used. On request, MMI can also provide custom made slides tailored to you particular experiment.

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tion of target molecules of diagnostic and therapeutic value", Clin. Exp. Metastasis 5 (24), 317 (2007).

6.2.1 Sample slides

Putting a droplet of a liquid with cells on a standard flat glass slide is the easiest way to prepare the source of cells for the capillary. The disadvantage is, that rather sizeable and thin liquid samples are formed which evaporate relatively quickly. There is a range of commercially available slides (e.g. IBIDI slides) that offer some advantages as compared to this simplest solution.

6.2.2 Target slides

Target slides can either be matrices of spots (hereafter called grids) or just a single spot. *Advalytix AmpliGrid* shown below is one of the standard grids predefined in the *mmi CellEctor* software. In addition, custom grids can be

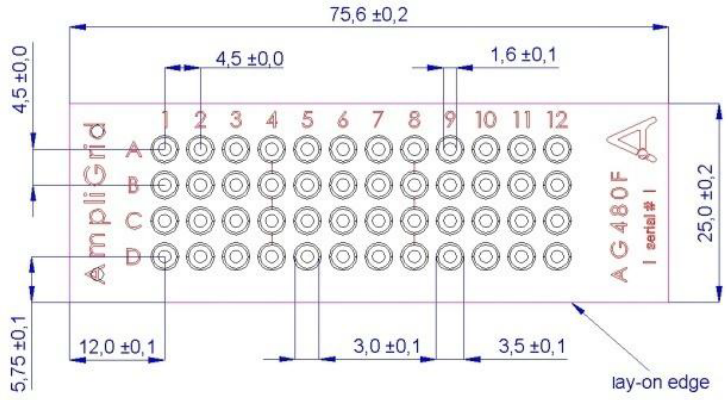


Figure 6.5: *The mmi CellEctor Advalytix AmpliGrid*

defined with up to 24 spots in arow or column (24 x 24 = 576 spots). A single spot as a target can be just a selected spot on a grid. MMI also offers a slide capable to hold a single transparent cap of a standard 0.2 ml PCR tube. This kind of the target slide is called single cap.

6.2.3 Service slide

On the service slide, the capillary can be treated with various solutions for cleaning, filling with additional reagents or diluting the reagents already present in the capillary. MMI offers IBIDI µ-Slide 8 well as the service slide.The slide can be purchased with different coatings (hydrophilic, hydrophobic, biopolymer or protein coating).

Figure 6.6: *The mmi CellEctor 8 well service slide*

6.3 Capillaries

6.3.1 Capillary types

mmi CellEctor standard glass capillaries are straight with the outer diameter of 1 mm and the tip inner diameter 10, 15, 20, 30 or 40 µm. The capillary tip is beveled at 45 deg. Any other capillary shapes and sizes that fit the capillary holder may also be used, however, in such a case the handling, the calibration and the standard usage procedures might differ from those described in this manual.

6.3.2 Capillary handling

Please observe the safety precautions described in the first chapter of this manual.

mmi CellEctor is delivered with a ready for use capillary placed in the capillary holder of the 3D manipulator (illustrated below).

The following capillary replacement procedure is recommended:

Figure 6.7: *The capillary (1) is mounted in a transparent capillary holder (2) that connects to the pump. The capillary holder is fixed in the manipulator holder (4) by a knob (3). An additional knob (5) allows changing the rotational position of the capillary holder. Furthermore, there is a screw at the rear side of the manipulator holder (4) that allows adjusting the capillary tilt. The standard tilt is 45 deg.*

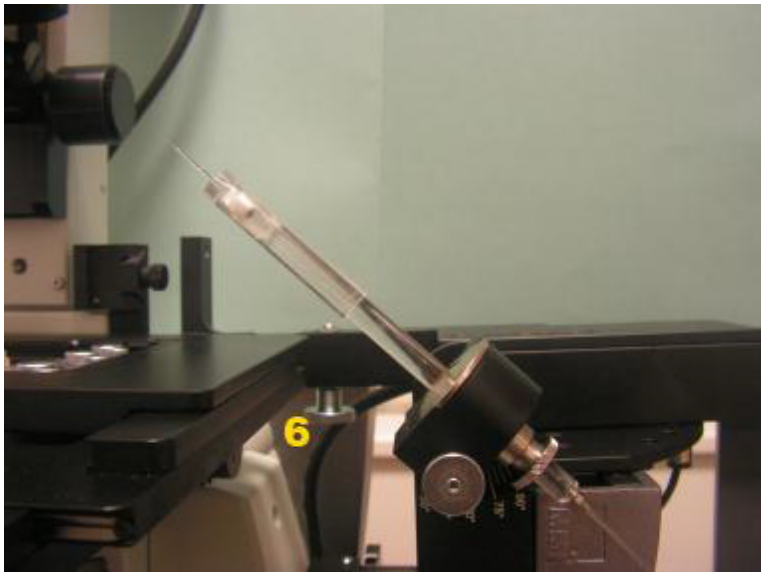


Figure 6.8: *The entire 3D manipulator can be tilted by 90 deg down to a position at which the replacement of the capillary is facilitated. For this purpose, unscrew the knob (6) and gently rotate the manipulator to the required position.*

Procedure

1. Place the 3D manipulator in the position shown in the figure 6.8.
2. Slide the capillary cover tube along the transparent capillary holder down to the lowest position.
3. While rotating the capillary cover tube, gently press it down until it steps into the screwing position.
4. In this position, slightly loosen the o-rings that tighten the capillary by rotating the capillary cover tube.
5. Pull the old capillary out of the holder.
6. Pump oil out until it reaches the outer o-ring that tightens the capillary. Take care than there are no air bubbles in the oil.
7. Insert the new capillary. While inserting, ensure that the capillary passes both o-rings.
8. Tighten the capillary by rotating the capillary cover tube.

At this stage, it is not recommended to fill the capillary with the oil since it is much easier to perform the 3D positioning when the capillary tip is empty. Once the capillary 3D positioning has been done, one can proceed with filling the capillary with oil and the buffer solution dedicated to collect cells. The following procedure is recommended:

Procedure

1. Fill almost the entire capillary with oil. The oil boundary must not enter the narrow part of the capillary, i.e. leave some air in the conical part of the capillary, see Fig. 6.9.
2. Stop pumping out and wait approx. 30 seconds for the pressure in the capillary to equilibrate.
3. Immerse the capillary into the buffer solution placed on a slide.
4. Pump in the buffer solution up to the moment the air gap between it and the oil moves into the transparent part of the grip head, see Fig. 6.10.
5. Stop pumping in and wait for the pressure in the capillary to equilibrate.
6. Move the capillary up from the buffer solution.

The air gap between the oil and the buffer solution works well for capillaries with the tip diameter of at least 30 µm. The narrower the capillary tip is, the longer time it takes to equilibrate the over- or underpressure after each pumping step which makes this method impracticable for smaller capillaries. In the later case it is recommended to collect cells in small volumes with the capillary filled with oil and air only.

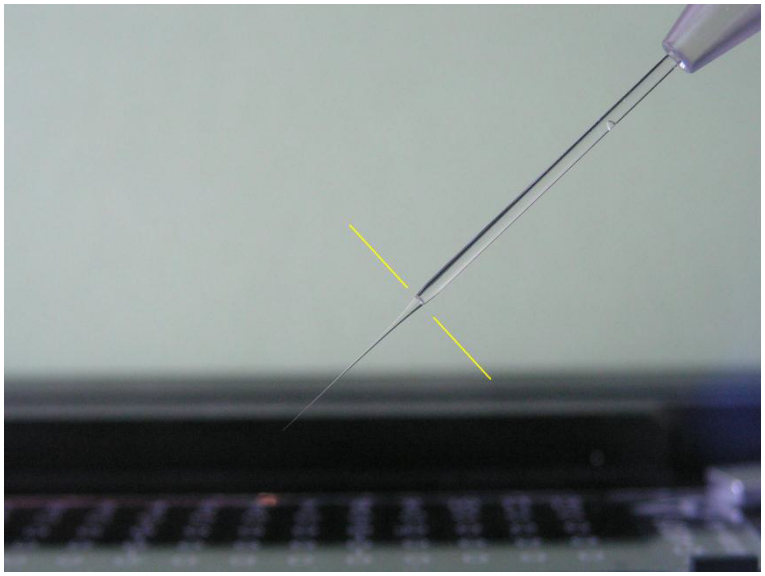


Figure 6.9: *Fill almost the entire capillary with oil. The oil boundary must not enter the narrow part of the capillary, i.e. leave some air in the conical part of the capillary.*

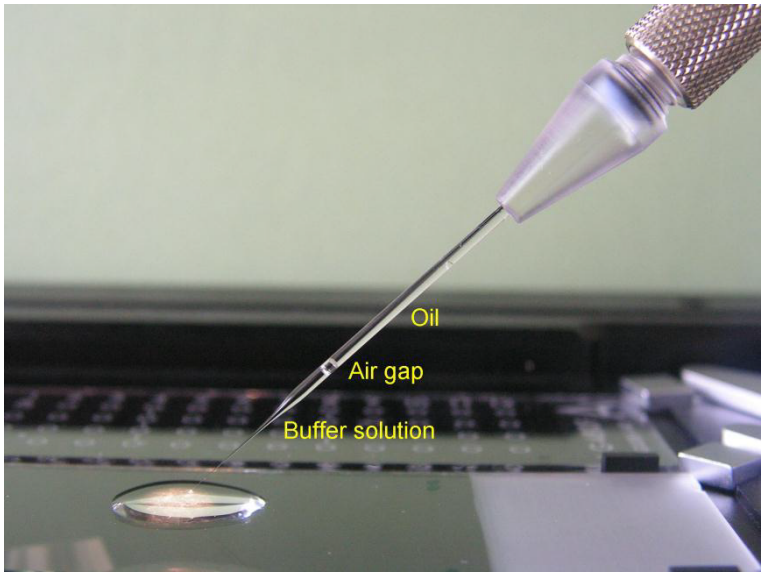


Figure 6.10: *Pump in the buffer solution up to the moment the air gap between it and the oil moves into the transparent part of the grip head.*

6.4 The *mmi CellPump*

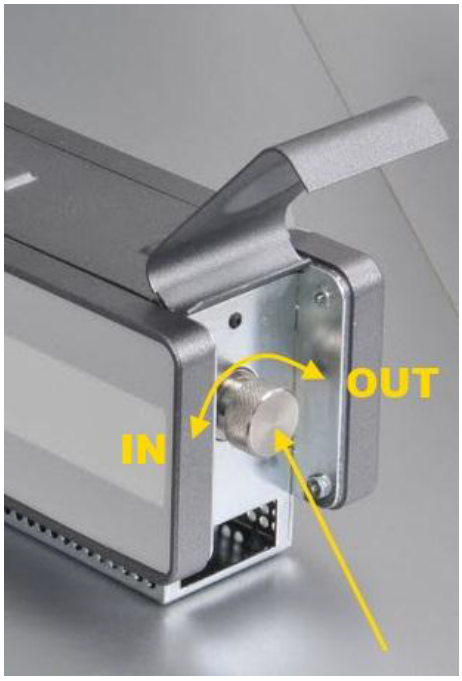


Figure 6.11: *The mmi CellPump allows to pump in and out by manually turning the rotation knob.*

Manual pumping The *mmi CellPump* can also be driven manually. For this, raise the rear cover of the pump and rotate the turning knob located there, see Fig. 6.11. Manual pumping is preferably used to fill the pump or the newly inserted capillary with the oil. While pumping manually, you must not move the pump piston outside the allowed range (see below).

> **While using capillaries with small opening, quick manual pumping may increase the pressure in the pump so that the capillary may burst.**

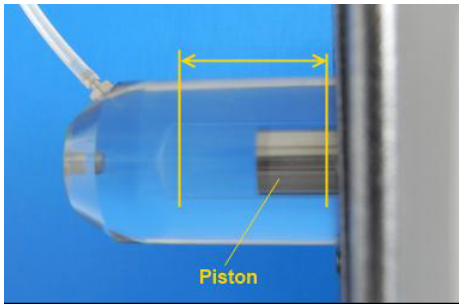


Figure 6.12: *The mmi CellPump allows to pump the shown range.*

Pumping range The pump piston has a limited range of movement as indicated in Fig. 6.12. If one of the limits has been reached and one tries to pump further

in the same direction using one of the buttons mentioned above, the software issues a warning like

Pump reached limit: pumpin IN impossible

In such a case, you have to move the piston in the opposite direction to get out of the end switch.

6.5 Use 3D manipulator with joystick

In *mmi CellEctor*, the joystick is used occasionally for the manual adjustment of the capillary position in 3D space. The horizontal XY position of the capillary can be changed by tilting the joystick to the left or right (X coordinate) or forth and back (Y coordinate). Its vertical Z position can be changed by turning the joystick clockwise for lowering (Z value increases) or anticlockwise for raising (Z value decreases) the capillary.

The speed with which the capillary moves is proportional to the deviation of the joystick from its equilibrium position. In order to get the feeling how quick the movement is, please observe the X, Y and Z current values in the advanced 3D capillary positioning dialog while using the joystick.

6.6 Typical workflow in collecting cells

While collecting cells, one picks a cell on the sample slide and deposits it on the target slide. The typical workflow for achieving this task with *mmi CellEctor* consists of the following steps:

- Ensure that the objectives are calibrated and the Z drive control is on (for automated microscope).
- Setup the *mmi CellEctor* major components: the slides (sample and deposit), the capillary and the pump.
- Calibrate the vertical contact point of the capillary on the sample and the deposit slide.
- Calibrate the capillary tip position on the screen on the sample slide.
- Working on the sample slide in the manual mode, adjust the capillary spacing and the pumping parameters so that the cells can efficiently be pumped in from the sample and pumped out on the deposit.
- Collect cells using either the automatic or the workflow mode.

The following chapters describe these steps in more detail. In a routine work under the same experimental conditions (same type and size of cells, buffer solution, capillary size, etc.), steps 1, 2 and 5 can usually be skipped.

6.6.1 Checking the microscope

Setting up the microscope for working with cells is described in detail in section 6.8 and 6.9. Here some measures you can apply to verify the setup:

- Camera orientation. Select the objective, you are going to work with, then move an object to the upper left screen corner so that the upper edge of the object touches the upper edge of the screen. Using the arrow key on the keyboard, move the object horizontally along the screen edge. The object should not change its distance to the screen edge while moving.
- Magnification factor for each objective. Click an object with the mouse and drag it into four corners of the screen. The object should not change its position with respect to the mouse cursor, see section 6.9.1.
- Parfocal lens offset (for automated microscope only). If it is correct, there should be no focus loss while changing the objectives, see section 6.9.2.
- Paraxial lens offset. If it is correct, there should be no displacement of markers with respect to the objects they mark while changing the objectives, see section 6.9.3.
- Mechanical limits. Ensure that the mechanical limits – which are always set on slide 1 – entirely cover the area of interest on the other slides, otherwise the stage may not reach some destinations on the latter ones, see section 5.5.1.

6.6.2 Setting up the slides, the capillary and the pump

- Type of slides. Decide what kind of slides will be used for the sample and the deposit. For the deposit, one can choose between AmpliGrid, custom grid or single cap, see section 6.8.5.
- Slide assignment. Place the sample and the deposit slides in the selected slide positions on the stage and assign the selected positions to the sample and deposit slides in the software, see section 6.8.1.
- Deposit configuration and calibration. For the grid deposits: tell the system the geometry of the grid (see section 6.8.5, custom grids only) and where the first spot of the grid is located on the microscope stage, see section 6.9.7.

- Capillary tilt. Insert a new capillary into the capillary holder, adjust its tilt and set the tilt value in the software, see section 6.3.2.
- Slide plane tilt. Select the working objective and determine the plane tilt of both slides, see section 7.4.2.

  Setting of the plane tilt does not apply to a single cap.
- Pump. Ensure that
  - the pump is filled with oil
  - the pump piston is away from its limit positions, see section 6.4
  - there are no air bubbles and no leaks between the pump and the capillary, see section 6.3.2

6.6.3 Calibrating the capillary

- Contact point. Determine the vertical reference position of the capillary, see section 6.9.4. On a flat slide, it is the position at which the capillary tip touches the slide surface.
- XY position on the screen. Once the contact point of the new capillary is known, move the capillary down and calibrate its XY position on the screen, see section 6.9.6.

6.6.4 Finding the optimum conditions for collecting cells

Solutions containing cells as well cells themselves exhibit a broad range of physicochemical and biological properties. Thus, the optimum conditions have to be found for the particular cells of interest. While experimenting with new cells and buffer solutions, one can vary the following parameters:

- Size of the capillary tip:
  In principle, it should be larger than the size of the cells to be collected. If the concentration of cells is not too large, even relatively large capillaries (tip diameter of 40 µm) can efficiently collect small cells (approx. 5 µm).
- Orientation of the capillary tip bevel:
  Rotating the capillary around its axis may affect the efficiency of catching and depositing the cells.
- Spacing:
  The distance between the capillary tip and the slide surface (spacing) affects both the volume of the liquid necessary to be pumped in order to pick a cell as well the volume to be pumped out at the deposit to get the cell out of the capillary. It should be adjusted for the sample and the deposit slide separately.

- Pumping parameters:
  These parameters have the major influence on the efficiency of collecting the cells. In most cases it is enough to adjust the main volume to be pumped in or out. In some extreme cases one has to use the other pump parameters like pre- and over-pumping, relaxation time or speed and acceleration of the pump, see section 6.8.3.

6.6.5 Performing cells collection

Cells can be collected using either the manual, automatic or workflow mode. In the manual mode, moving the stage, moving the capillary up or down and pumping are action separately launched by the user. This mode is suitable learning the *mmi CellEctor* and for finding the optimum conditions. The routine work is preferably done in the workflow mode that combines these single actions in series of actions, see section 6.6.

6.7 *mmi CellEctor* user interface

As described in section 5.1, the special features of the mmi CellCut, the mmi CellExplorer and mmi CellManipulator are installed as separate plug-ins (software modules). The mmi CellManipulator plug-in appears as a separate tool panel on the right side of the program window.

To switch from one plug-in to the other you only have to click on the appropriate tool panel, see Fig. 6.13. Once the *mmi CellEctor* tab is selected in the component control panel of the *mmi CellTools* main window, *mmi CellEctor* becomes the active component. It adds its own menu items grouped as *CellEctor* to the main menu and shows its own control panel, either System or Action panel.

6.7.1 Menu

The CellEctor menu consists of two sub-menu groups

Configure

and

Calibrate

The menu item Export grid data shows up only if AmpliGrid or custom grid is selected as the deposition target.

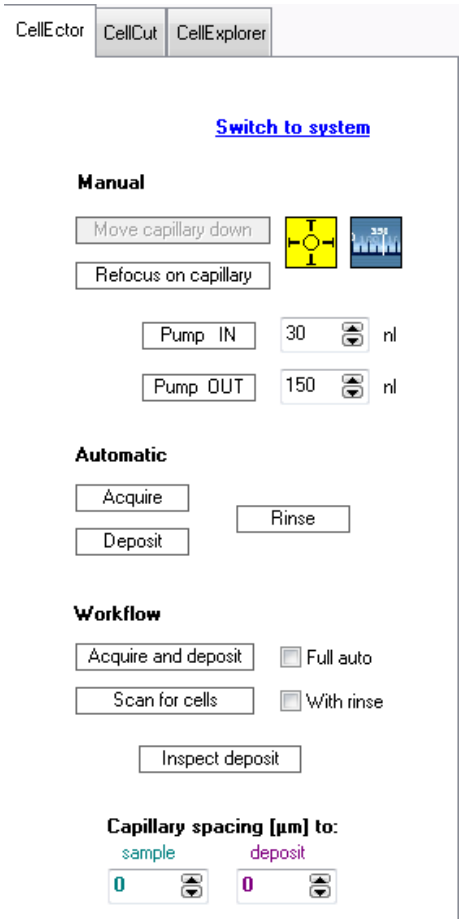


Figure 6.13: *The mmi CellEctor plug-in panel in action mode*

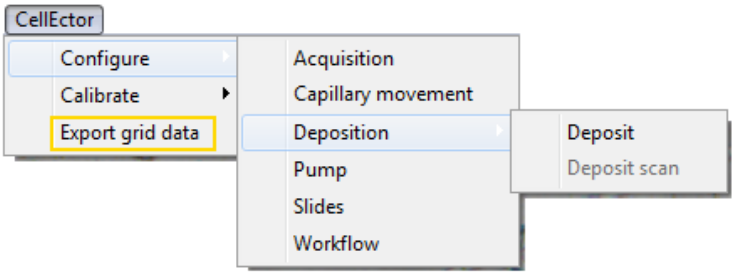


Figure 6.14: *The mmi CellEctor menu*

6.7.2 System panel

There are two major panels in the *mmi CellEctor* control panel: System panel and Action panel. You can switch between the panels by clicking on the links in the top right corner. The System panel shows the readiness of the major system components. The figure below shows the system ready for use. If one of the major system components requires calibration, the corresponding button is highlighted red. Clicking the button with the left mouse key launches

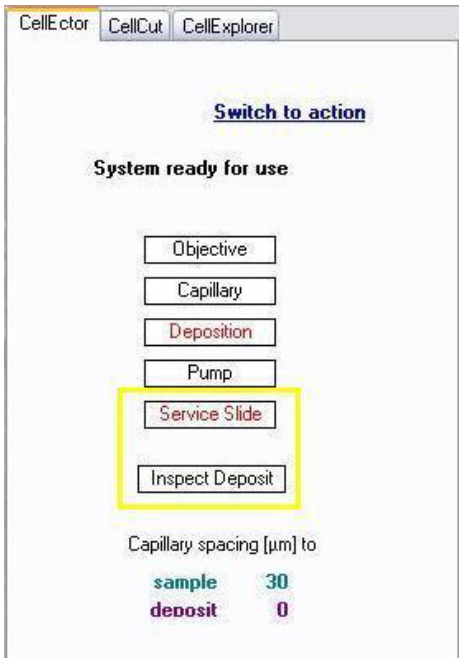


Figure 6.15: *The mmi CellEctor plug-in panel in calibration mode*

the calibration procedure of this component. Clicking the button with the right mouse button launches the configuration dialog of this component, if the corresponding item exists in the CellEctor main menu. Clicking the link **Switch to action** opens the action panel.

> The button Inspect Deposit shows up only for grids as deposition targets and the button Service Slide only for non-standard deposition targets.

6.7.3 Action panel

Most of the routine work with the *mmi CellEctor* is done using the Action panel. It consist of the four groups of controls:

- Manual operation
- Automatic operation

- Workflow
- Capillary spacing

Here, the controls will only briefly be explained.

- Move capillary down/up. Moves the capillary down to the point and in the way defined in the capillary calibration and configuration procedures. Once the capillary is down, press the button again to move it up to the idle position.
- **Refocus on capillary/slide**. This button toggles the focus between the capillary tip and the slide surface. NOTE. The button shows up only if the Z drive control of the automated microscope is on.
- **Marker button** Changes the mouse cursor to a cross with which you can mark a cell to be acquired.

- **Measure button** Changes the mouse cursor to a hand with an arrow with which you can measure the size of the objects in the video panel.

- **Pump IN** and **Pump OUT** Pressing these buttons launches the relevant pumping action.
- **Volume IN/OUT** Two spin boxes that allow to adjust the amount of the liquid pumped in or out, respectively.
- **Acquire** Launches the acquisition action that consists of the following steps:
  1. Moving a marked cell to the capillary down position
  2. Moving the capillary down
  3. Pumping in
  4. Moving the capillary up
- **Deposit** Launches the deposition action that consists of the following steps:
  1. Moving the stage to the deposit slide
  2. Moving the capillary down
  3. Pumping out
  4. Moving the capillary up
- **Rinse** Launches the workflow to be done solely on the service slide.
- **Acquire and deposit** Acquisition and then deposition are launched. The user has to mark a cell before pressing this button. If the check box **With rinse** is checked, the capillary treatment sequence on the service slide is included. It means that the entire workflow as defined in the workflow editor table will be done, see chapter 6.8.6. if the check box **Full auto** is checked, the workflow starts immediately after selecting a cell with a marker.

- **Scan for cells** starts scanning of the predefined sample area, cells recognition, acquisition, deposition and rinse, with the latter being performed only if the With rinse check box is checked.
- **Capillary spacing** Two spin boxes that allow adjusting the distance between the capillary tip and the surface of the sample or deposit slide.

  The values in these two boxes are in effect before the capillary starts its movement down. For changing the spacing when the capillary is already down use the fine positioning arrows shown below.

- These two arrows show up as soon as the capillary arrives to the down position. Pressing one of them moves the capillary up or down in fine steps giving the opportunity to adjust the capillary spacing. The spacing value in the corresponding spin box is automatically adjusted and is then used in the future movements down.

  Once the fine adjustment arrows show up, the relevant spin box is disabled.

- **Rough spacing mode warning** shows up when the Z drive control of the automated microscope has been turned off or you are working with the manual microscope. It indicates that the numbers in both spin boxes are approximate.

Like in the system panel, clicking some buttons (capillary, pump, acquisition, deposition, rinse) with the right mouse key launches the relevant configuration dialogs.

If some system components are not calibrated, the affected buttons in the Action panel are temporarily disabled(grayed).

The link **Switch to system** opens the system panel.

6.7.4 Distance measurements

Pressing the Measure button the Action panel changes the mouse cursor to a hand with an arrow that allows measuring the distances in the video panel. For this, just drag the cursor from the start to the end point of the interest and the distance between these two points will be indicated on the screen. Every subsequent measurement deletes the previous one. Once you are done with the measurements, press the measure button again (which is now shown as an arrow) to change to the cursor default shape.

6.8 Configuration routines

Before you start to use *mmi CellEctor* for the first time, some preliminary work is necessary to make the system ready for use. Most of this work has to be done only once and is usually done by the MMI service technician during the installation of the system. Some activities are recurrent, like capillary 3D positioning of a freshly inserted capillary.

6.8.1 Choosing the position of sample, target and service slide

The multi-slide holder of the stage can hold up to three slides of the standard size. One can change the slide assignment in the menu item

CellEctor →*Configure* →*Slides*

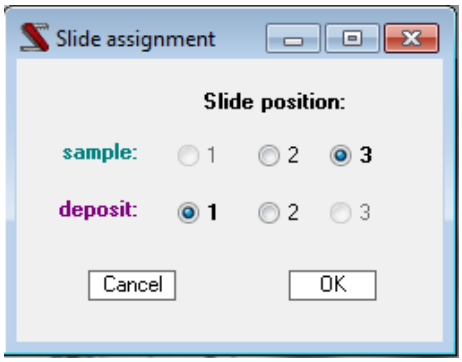


Figure 6.16: *The slide configuration window to select the position of the sample, target and service slide.*

The picture above shows the default slide assignment. The sample and the deposit slides can be placed at arbitrary positions. The third unoccupied position is reserved for the service slide.

> Once you change the position of the slides, re-calibrations are necessary (capillary, deposit, plane tilt). Thus, it is recommended to change the slide assignment before you start calibrations while preparing the system for use.

6.8.2 Capillary movement configuration

The capillary tip moves from the idle position towards the destination point along the three vectors shown in Fig. 6.17:

- A – horizontal movement

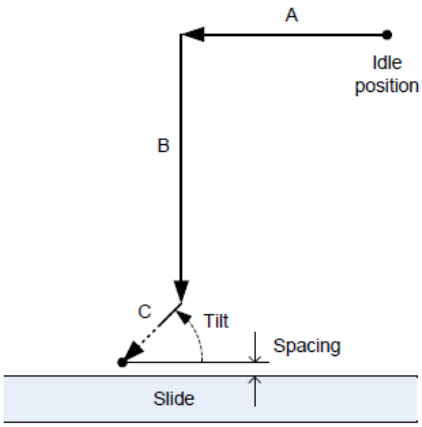


Figure 6.17: *Movement pathway of the capillary from idle to down position: A – horizontal movement; B – vertical movement; C – axial movement (immerse distance)*

- B – vertical movement
- C – axial movement (immerse distance)

The user can adjust the movement characteristics in the following dialog (Fig. 6.18) that can be opened from the main menu

CellEctor →Configure →Capillary Movement

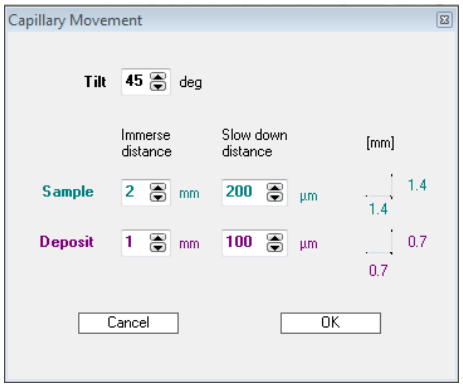


Figure 6.18: *Capillary movement configuration dialog.*

The **tilt** of the capillary should be set equal to the value read from the capillary holder. **Immerse distance** corresponds to vector C in the above figure. **Slow down distance** is the last portion of the immerse distance (indicated as a dashed line on vector C in the above figure) where the capillary moves with a highly reduced speed in order to minimize pushing away the cells.

While moving up out of the sample, the capillary moves with the normal speed.

Both immerse as well as the slow down distance can be set for the sample and the deposit independently. The numbers at the right hand side of the dialog (in [mm]) are calculated from a given capillary tilt and a given immerse distance. They help to adjust the immerse distance if the capillary has to be immersed in liquids confined to container walls.

6.8.3 Pump configuration

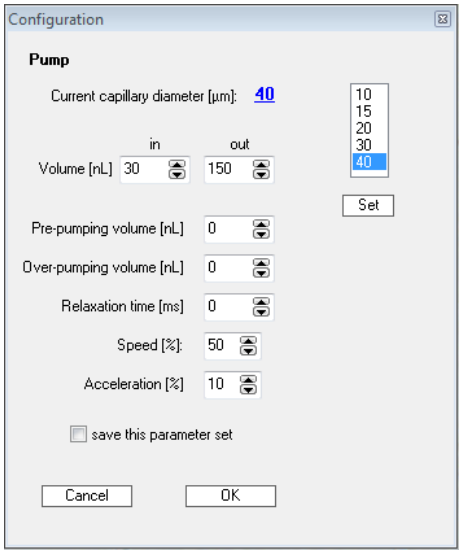


Figure 6.19: *Pump configuration dialog.*

All the pump parameters can be set in the pump configuration dialog that can be opened either from the main menu

CellEctor → *Configuration* → *Pump*

or by clicking any pump-relevant button with the right mouse button. The following pump parameters can be changed by the user:

- **Volume in** main volume that is pumped in if the user presses the button Pump in or Acquire in the action panel
- **Volume out** main volume that is pumped out if the user presses the button Pump out or Deposit in the action panel
- **Pre-pumping volume** volume that is added to the main volume whenever the pump changes the direction (from in to out or vice versa)
- **Over-pumping volume** volume that is pumped in prior to the main volume to be pumped in and then pumped out after the main volume has been pumped in. This value has no effect on the pumping out process.

- **Relaxation time** waiting time spent for pressure to equilibrate after the pumping process is finished
- **Speed pump** speed (in percent of the maximum speed)
- **Acceleration pump** acceleration (in percent of the maximum acceleration)
- **40** number showing the current capillary size for which the pump parameters are displayed. Click on it opens the list with available capillaries (as shown in the figure above).

All the pump parameters are specific for a given capillary. Up to five parameter sets can be stored for capillaries with different sizes. You can retrieve a stored parameter set for a given capillary by selecting the corresponding capillary size.

> Whenever the pump parameters are modified, the changes are valid for the running session only. In order to save the current set for the future sessions, check the box **Save this parameter** set before you leave the above dialog.

6.8.4 Acquisition configuration

One can adjust some parameters of the acquisition process in the configuration dialog that can be opened either from the main menu

CellEctor $\rightarrow$*Configure* $\rightarrow$*Acquisition*

or by clicking any acquisition-relevant button with the right mouse button. In the dialog showed in Fig. 6.20, the upper part **Acquisition** defines the behavior of the single acquisition process, the lower part **While scanning for cells, scan** that of the fully automated cell acquisition. Acquisition mode is presently limited to single cells. This means that the user cannot set more than one marker at a time to select a cell and to acquire it. However, several cells may be collected in the capillary by repeating the acquisition step before the deposition of the cells takes place. In such a case, one has to adjust the volume that is pumped out during the deposition process to at least multiple of the volume pumped in during a single acquisition step in order to ensure that all the collected cells will leave the capillary upon deposition. Two other options allow further modification of the acquisition process:

- **Allow for acquisition without markers** if this option is checked, the system does require setting a marker to acquire a cell. If the option is unchecked a message is issued when the user clicks **Acquire** button without setting a marker prior to it.

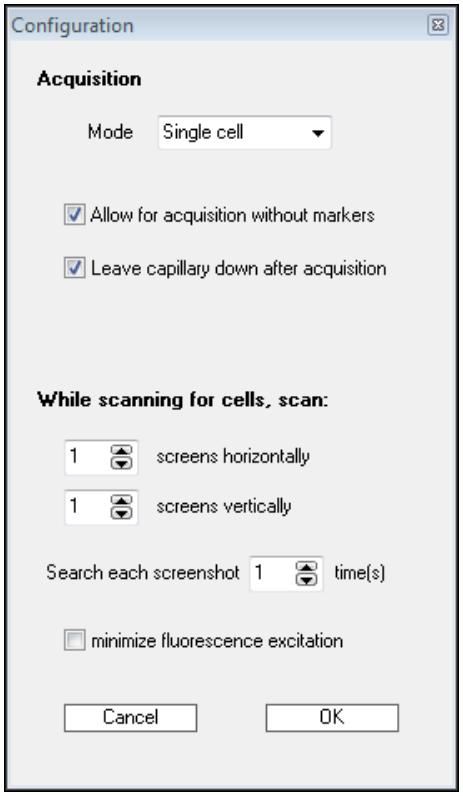


Figure 6.20: *Cell acquisition configuration dialog.*

- **Leave capillary down after acquisition** check this option if you want to verify that the cell has indeed been caught in the capillary during the acquisition process. If it has, you can click **Deposit** button to move the capillary up and continue the process. If it has not, you have the opportunity to retry pumping the cell into the capillary by pressing **Pump IN**.

  Whenever the capillary is down, movement of the XY-stage is still possible. However, please be aware that large movements (exceeding the size of the field of view) may damage the capillary when the slide plane is significantly tilted.

These options are especially useful during the fine tuning of the acquisition process for a given application. In the workflow mode **Scan for cells** acquisition is preceded by making a snapshot of the current field of view and automatic cell recognition and selection (setting markers). The sample is scanned horizontally and vertically and the user can define the number of screenshots that have to be made (scan a **number of screens horizontally and a number of screens vertically**). The recognition of cells can be repeated a number of times at each stage location if there are several cells of interest that can be acquired there. **Minimize fluorescence excitation** option is relevant only for the case when the automatic cell recognition takes place in the fluorescence

observation mode and one wants to prevent bleaching of cells. If this option is checked, the motorized fluorescence shutter is opened just before a screen-shot is made and closed immediately afterwards.

6.8.5 Deposition configuration

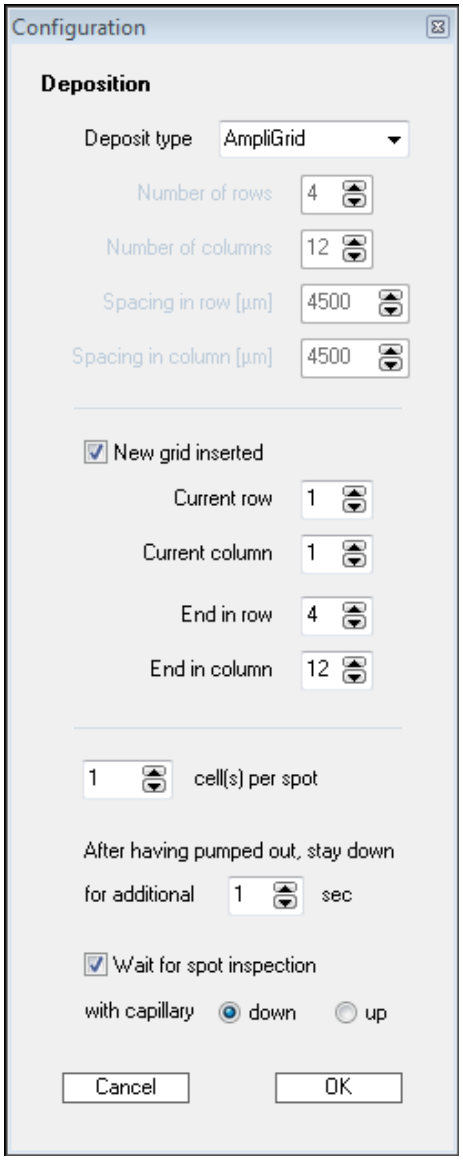


Figure 6.21: *Cell deposit configuration dialog.*

One can adjust several parameters of the deposition process in the configuration dialog that can be opened either from the main menu

CellEctor → *Configure* → *Deposition* → *Deposit*

or by clicking any deposition-relevant button with the right mouse button, see

Fig. 6.21. The choice of the deposit type has effect on many other settings of the system. Presently, one can choose among three types of the deposit:

- **AmpliGrid** commercially available grid
- **Custom grid** grid defined by the user
- **Single cap** transparent cap placed in a holder

If the user changes the deposit type, the configuration parameters irrelevant to the selected type are disabled. For instance, the number of rows and columns as well the spacing are not applicable to the single cap. Changing the type also deletes all the settings and data associated with the current deposit target and the check box **New grid(cap) inserted** is automatically checked. The basic parameters of a grid (rows, columns, spacing) can be changed only for a custom grid. On the other hand, the number and the location of the target spots can be selected arbitrarily on both AmpliGrid as well as on the custom grid by changing the **current row**, the **end in row**, the **current column** and the **end in column**.

The number of cells per spot is used by the software to determine whether or not a given spot is already filled and the stage has to be moved to the next spot. No further deposition is possible if all the spots of a grid are filled with the pre-defined number of cells. The same applies to a single cap.

The remaining three options facilitate the visual control of the deposition of cells during the automatic acquisition and deposition. In the standard deposition process, the capillary is moved up as soon as the pumping out step is completed (including the pump relaxation time). This corresponds to N = 0 set in the spin box **After having pumped out, stay down for N seconds**. Change this number if you want the capillary be left down for an additional amount of time (maximum 20 seconds) before it is moved up and the XY-stage moved back to the sample. This number becomes irrelevant if the option **Wait for spot inspection** is checked. In this case the XY-stage is not moved back to the sample until the user clicks **Back to sample** from the context menu on the video image.

6.8.6 Workflows editor

In *mmi CellEctor* a workflow is a combination of acquisition, deposition and the capillary treatment on the service slide that is launched by a single mouse click as a continuous series of actions. The workflow can be customized in the following workflow editor, see Fig. 6.22.

In the example shown in the above figure, the workflow consists of four consecutive steps:

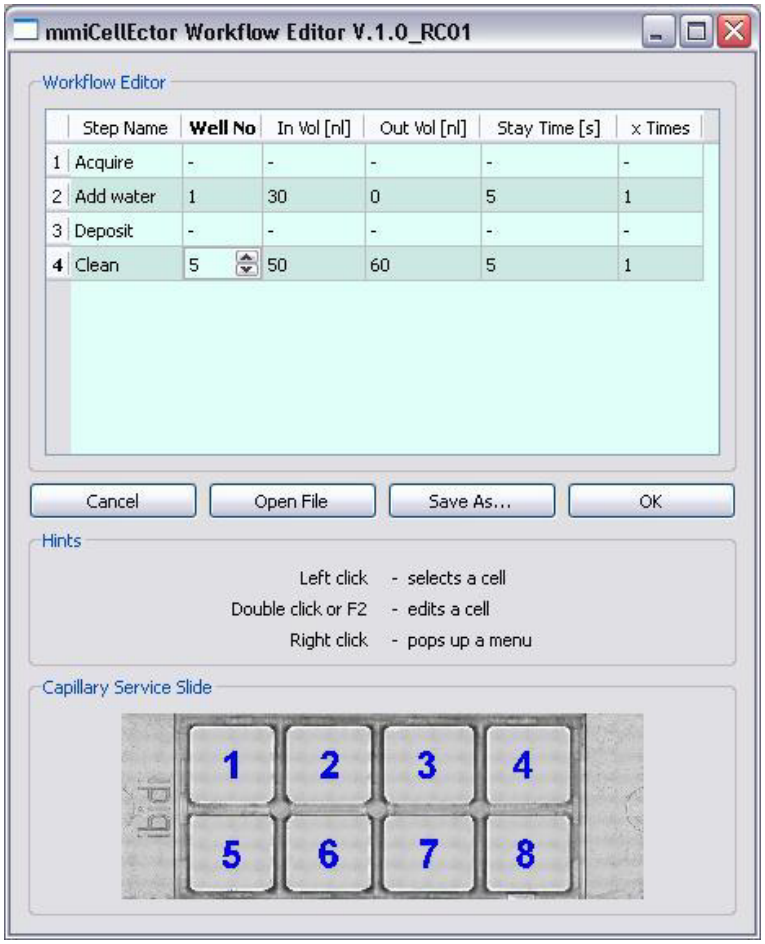


Figure 6.22: *The workflow editor*

Procedure

1. acquisition
2. diluting the reagent in the capillary by adding some water placed in well #1 of the service slide
3. deposition
4. cleaning the capillary in well #5 of the service slide

The parameters of a single step can be modified by editing the values in a single row of this table. The entire rows can be deleted, inserted or appended using the commands from the pop-up menu, see Fig. 6.23 The parameters in the rows **Acquire** and **Deposit** cannot be changed and these rows cannot occur more than once in this table (that is why the menu items

Insert →Acquire and Insert →Deposit

are disabled in Fig. 6.23).

Figure 6.23: *The workflow editor*

Once the workflow has been edited and the workflow editor closed, it becomes the active workflow in *mmi CellEctor*. Depending on which button is pressed in the action panel, either the entire workflow or only its part is launched. For example, pressing the button Rinse launches the actions on the service slide only ("Add water" and "Clean" in the above example). Pressing **Acquire and deposit** button with the check box **With rinse** checked launches the entire workflow, otherwise only the acquisition and deposition steps are performed.

The workflow editor can be opened by either selecting the corresponding menu

CellEctor → *Configure* → *Workflow*

or by pressing the right mouse button over the relevant buttons **Rinse**, **Acquire and deposit**.

A running workflow can be interrupted any time by pressing the **ESC** key on the keyboard. In such a case, the workflow terminates after the currently running basic step is completed (capillary movement, pumping, moving the stage). If a workflow is on idle, i.e. waiting for the next step a predefined amount of time (**Stay time** in the workflow editor), pressing ESC terminates the workflow immediately.

6.8.7 Deposit inspection

The deposit inspection calibration can be launched by the menu item

Cellector → *Configure* → *Deposition* → *Deposit scan*

or pressing the **inspect deposit** button with the right mouse button opens the configuration dialog shown below. The scanning can involve either the entire grid (check the box Scan entire grid) or only a part of it. For the latter, define the starting and the ending row and column.

For visual inspection, define the number of seconds the XY-stage will stay at each spot.

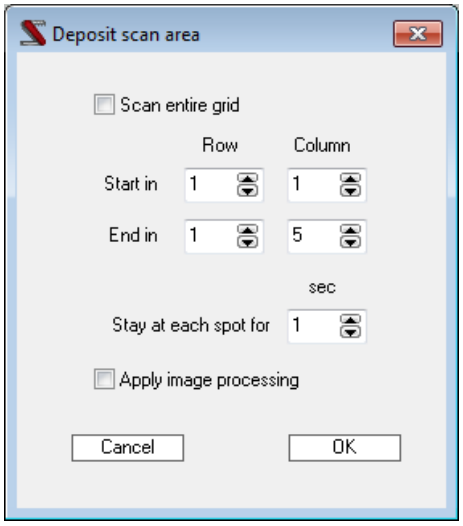


Figure 6.24: *Configure inspection of the deposit slide.*

For automatic inspection of the spots, check **Apply image processing**. In this case the XY-stage waits until the recognition of cells is completed by *mmi CellExplorer*. A report will be created automatically, also see chapter 6.10.6.

> Prior to starting the deposit inspection with the image processing, the correct image processing parameters have to be loaded to *mmi CellExplorer*.

6.9 Calibration routines

In order to efficiently perform the acquisition and deposition of cells, *mmi CellEctor* requires the microscope objectives be set up properly. The objective setup includes the following

Procedure

1. XY calibration of the objectives (magnification)
2. calibration of the parfocal lens offset (automated microscope only)
3. calibration of the paraxial lens offset

6.9.1 Objectives magnification

When the mouse is dragged within the video panel in the movement mode, the stage moves by a distance depending on the magnification of the objective in use. Thus, a calibration of the X and Y coordinates is necessary for each objective separately. To perform the XY calibration of an objective, select

CellEctor →*Calibrate* →*Objective* →*Magnification*

from the main menu or press the button Objective in the CellEctor system panel.

Several things happen now:

- two transparent rectangles show up in the top left and the bottom right video panel corner
- mouse cursor changes to a target shape
- objective calibration panel shows up

The objective calibration panel guides you through the following calibration steps:

Procedure

1. Press the spacebar to switch between drawing and moving mode and move a distinct object to the rectangle in the top left corner of the video panel. The object does not need be centered within the rectangle.
2. Toggle the cursor back to the target shape and click theobject with the left mouse key. This marks the object with the target marker.

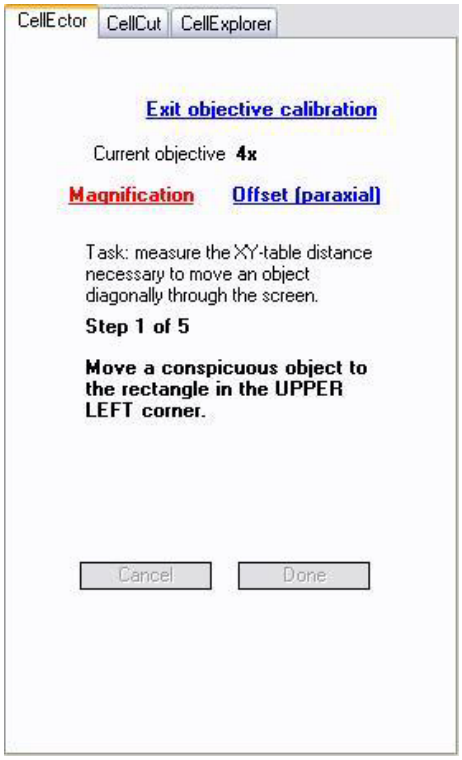


Figure 6.25: *The mmi CellEctor plug-in panel in objective calibration mode*

3. Toggle the cursor back to the hand shape and move the object to the bottom right rectangle.
4. Toggle the cursor to the target shape and mark the object again.
5. Click Done on the objective calibration panel to finish, or Cancel to restart the procedure.

After a successful calibration, any object dragged to any location within the video panel should be exactly under the mouse cursor at the end of dragging.

Whenever an objective is re-calibrated, re-calibration of the XY-position of the capillary as well as of the deposit is necessary. This is indicated by marking the Capillary and the Deposition buttons red in the CellEctor system panel

6.9.2 Parfocal lens offset

This procedure applies to the automated microscope only.

When one changes an objective and the microscope was in focus, the new objective requires a slightly different Z drive position to get in the new focus.

The difference is called parfocal lens offset. The *mmi CellTools* software can automatically refocus after a change of the objective if the offset has been determined for all the objectives in use. Proceed as follows in order to calibrate the parfocal lens offset:

Procedure

1. Change to the objective with the highest magnification
2. Focus the microscope
3. Change to the objective with the next smaller magnification
4. Focus the microscope
5. Select menu item *Microscope →Z Drive Offset →Objective (parfocal)*
6. Repeat steps 3 and 4 for the remaining objectives.

After the calibration, the microscope does not lose focus when the objectives are changed.

6.9.3 Paraxial lens offset

Due to mechanical and optical tolerances, two objectives never have exactly the same optical axis. The difference between the position of the optical axes of two objectives is called paraxial lens offset. This offset manifests itself by a small shift of pixels in the video panel when objectives are changed. The *mmi CellTools* software can automatically compensate this shift if the paraxial lens offset has been determined for each objective in use. To perform the calibration of the paraxial lens offset, select

CellEctor →Calibration →Objective →Lens Offset (paraxial)

from the main menu or press the button Objective in the CellEctor system panel and then click the link Offset (paraxial). In the lens offset calibration procedure, the XY displacement of a sizeable object is measured while changing the objectives from the highest to the lowest magnification. The panel shown below guides you through the following calibration steps:

Procedure

1. Change to the objective with the highest magnification and/or click Start button to confirm it.
2. Two tool buttons show up:

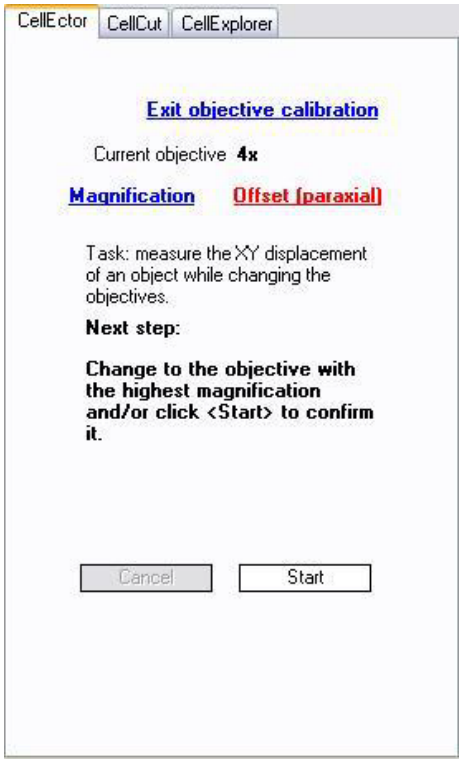


Figure 6.26: *The mmi CellEctor plug-in panel in objective paraxial calibration mode*

- freehand drawing tool
- drawing mover tool

Move the selected object to the center of the video panel, click the freehand drawing tool and draw the contour of the object with the mouse. Press Del on the keyboard if you want to redraw the contour.

3. Change to the objective with the next smaller magnification

4. After the change, the previously drawn contour will move away from the object. Re-position it using the drawing mover tool so that it again overlaps with the object.

5. Click the button Set offset and confirm the message that shows up

6. Repeat steps 3 through 5 for the remaining objectives

7. Change from the objective the smallest to the objective with the highest magnification without deleting the drawn shape. If the procedure succeeded, the shape should overlap with the object.

8. Terminate the procedure by clicking the link **Exit objective calibration** on the panel

6.9.4 Capillary vertical contact position: automated microscope

> The procedure for the determination of the vertical contact position described in the first part of this chapter applies to the automated microscope only. A simplified method for manual microscopes is described at the end of the chapter.

Whenever a new capillary is inserted, one has to calibrate the capillary in two steps:

- Step 1. Define the vertical reference position of the capillary in the contact with the (planar) slide.
- Step 2. Define the XY position of the capillary tip on the screen.

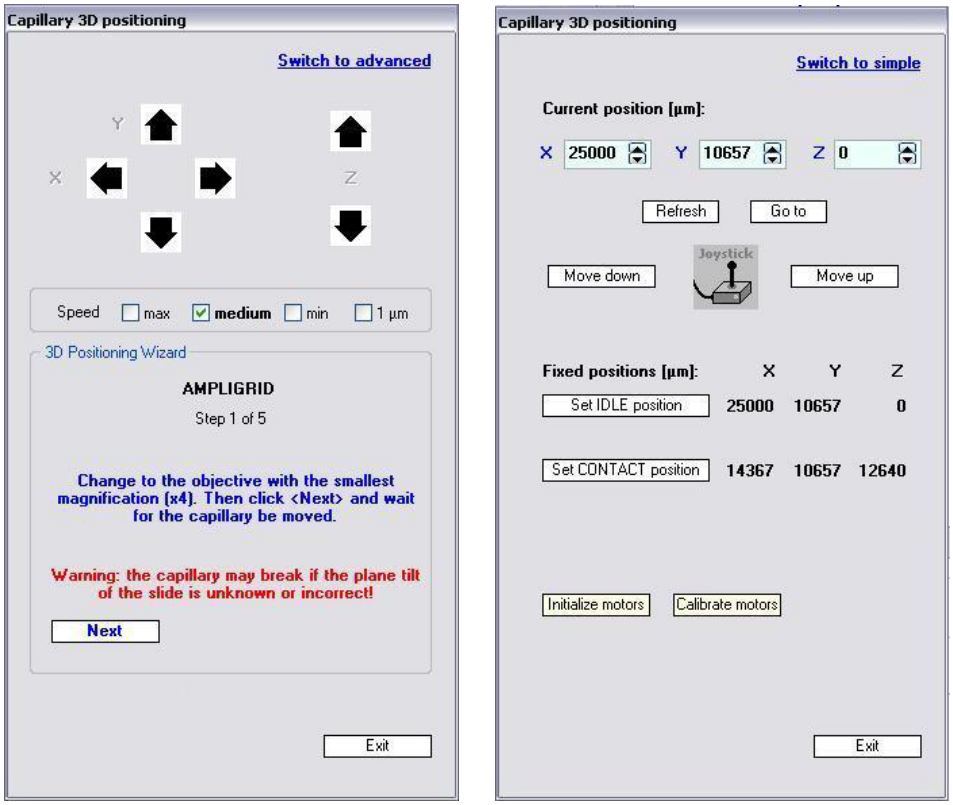
Defining the vertical reference position of the capillary at the contact with the slide is done in the 3D positioning dialog accessible through the link **Switch to system** or the menu

> *Cellector* → *Capillary*

and than click on the button **Contact Point**. One can choose between the simple, see Fig. 6.27a and the advanced positioning method, see Fig. 6.27b. In the simple method, a step-by-step wizard guides you through the calibration and there is no need to use joystick. This method is recommended for most users. The advanced method is recommended for those who prefer to work with joystick and/or numbers. As both methods result in the same accuracy of the positioning, it does not matter which one you actually use. The wizards for the simple method differ slightly depending on whether the calibration has to be done on a flat slide or in a cap.

> Neither of the methods will work properly if the plane tilt of the slide is unknown or incorrect.

The simple method on a flat slide runs through 5 steps described below and has to be done on both the sample as well the deposit slide in a deliberate order. Just carefully follow the instructions (marked blue) and warnings (red) in the wizard and perform the required actions. For documentation purposes, the detailed explanation is given below.



(a) Simple method. The wizard only shows up, if plane tilt is active.

(b) Advanced method. Fixed positions only show up, if plane tilt is active.

Figure 6.27: *Capillary vertical contact point calibration windows.*

Capillary vertical contact position: Ampligrid or custom grid, simple method.

Procedure

1. Once the 3D positioning dialog is opened, a large target marker is shown in the center of the screen facilitating the positioning. At this moment, the

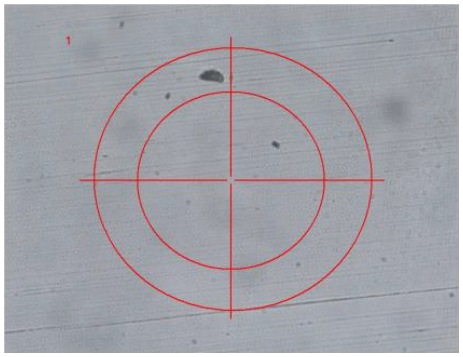
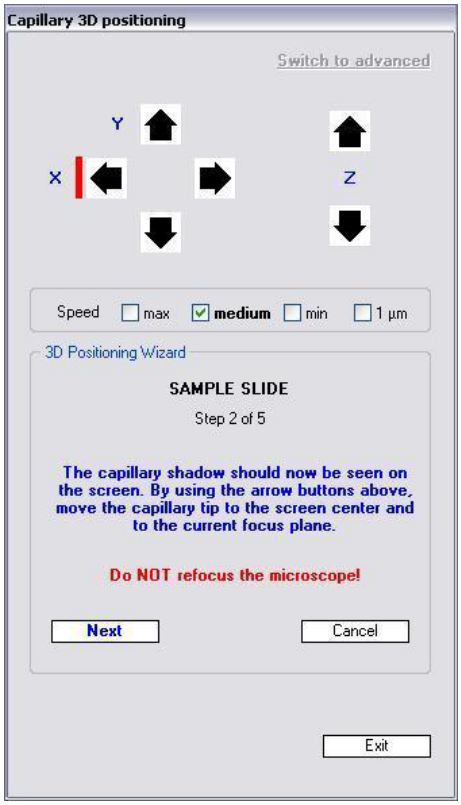


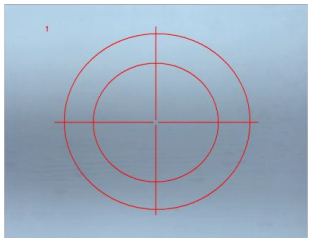
Figure 6.28: *Center of the screen indicated in the video panel.*

capillary stays in the idle position which is at the maximum distance from the slide. This is indicated by a red bar above the Z arrow (no further raising of the capillary is possible). The system is now ready to move the capillary towards the optical axis of the objective and to lower it towards the slide. The first step is to change to the objective with the smallest magnification, usually x4. Then press **Next**.

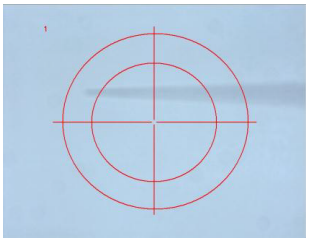
2. The capillary reached the leftmost X position (note the red bar at the X arrow in Fig. 6.29a) and was lowered so that its shadow should already be seen in the screen, see Fig. 6.29b. If it is not, you have to adjust its horizontal position first. Using the X and Y arrows, place the capillary tip



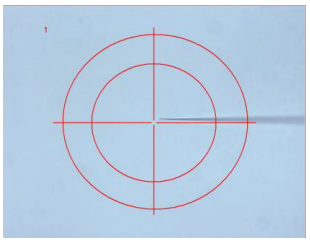
(a) The control panel.



(b) Capillary moved over the center marker but is still above the optical plane.



(c) Capillary lowered to the optical plane.

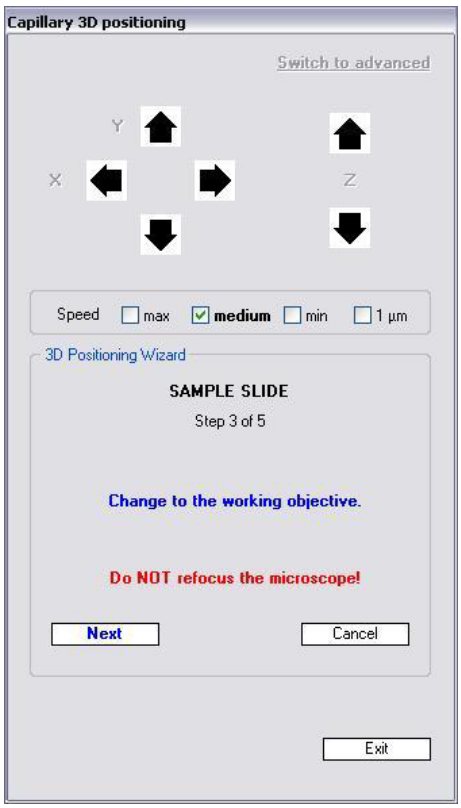


(d) Capillary positioned exactly on the center marker and to the optical plane.

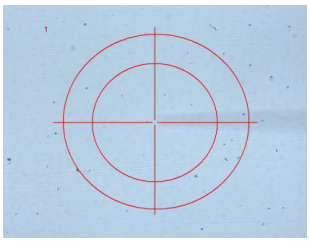
Figure 6.29: *Step2 of capillary vertical positioning wizard.*

in the center of the target marker, as shown in Fig. 6.29c. Then, move the capillary down or up using the Z arrows so that its tip is in focus like in Fig. 6.29d. Then press **Next**.

3. The system has now set the focus from the capillary tip back to the slide. Change to the objective you anticipate to work with during collection of cells. Then press **Next**.



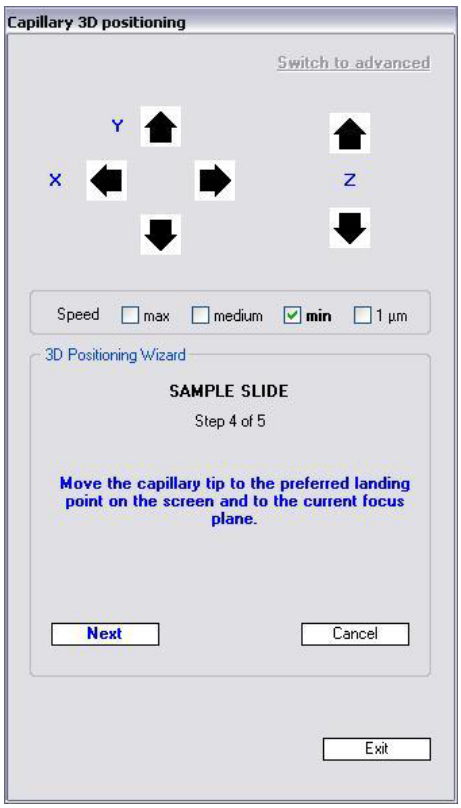
(a) The control panel.



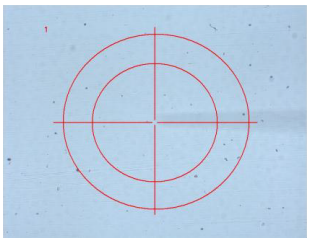
(b) The system has now set the focus from the capillary tip back to the slide.

Figure 6.30: *Step3 of capillary vertical positioning wizard.*

4. The system further lowered the capillary, thus it is roughly in focus again, see Fig. 6.31b. The XY position of the capillary tip on the screen can de-



(a) The control panel.

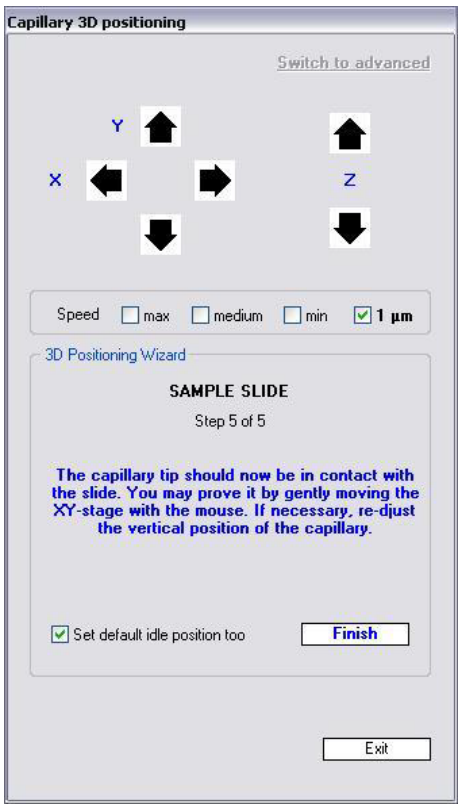


(b) The system further lowered the capillary, thus it is roughly in focus again.

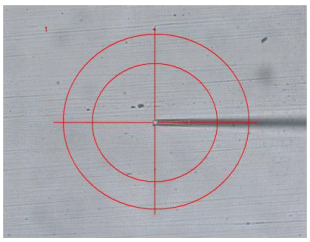
Figure 6.31: *Step4 of capillary vertical positioning wizard.*

liberately be chosen, i.e. does not have to be in the screen center anymore. You can adjust it using the X and Y arrows. The vertical position of the capillary must be adjusted so that its tip gets in a gentle contact with the slide. Use the Z arrows and the minimum speed to change the vertical position. Then press **Next**.

5. At the contact position, both the slide and the capillary tip should be in focus, as illustrated in Fig. 6.32b. Press **Finish** to set the contact



(a) The control panel.



(b) At the contact position, both the slide and the capillary tip should be in focus.

Figure 6.32: *Step5 of capillary vertical positioning wizard.*

point of the capillary tip with the slide and to complete the capillary 3D positioning.

> It is recommended to leave the check box **Set default idle position too** checked. The default idle position is defined as the maximum XZ distance from the sample while leaving the Y coordinate unchanged. Setting user-defined idle position of the capillary is possible in the advanced 3D positioning only.

Once Finish is clicked and the wizard completed, the following dialog Fig. 6.33: If the contact position of the capillary on the other slide is still valid, i.e. you

Information request
Please select:
the contact position on the DEPOSIT is still valid
I will set the contact position on the DEPOSIT in the next step
OK

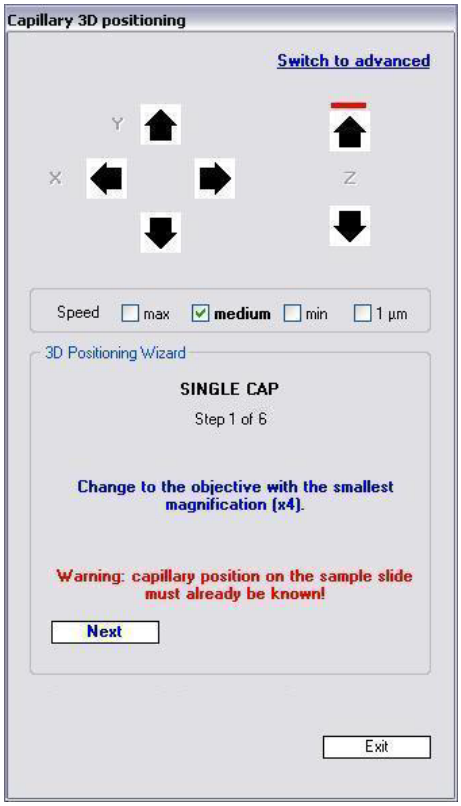
Figure 6.33: *Dialog at the end of the capillary vertical position calibration wizard.*

have replaced neither the capillary nor the other slide, select the option "still valid". Otherwise select the second option, move to the other slide (in this case to the deposit) and go through the calibration procedure once again.

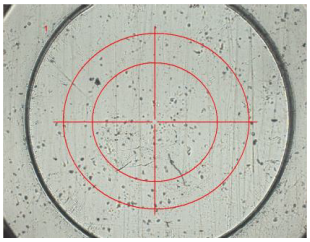
Capillary vertical contact position: Single cap, simple method The wizard for a single cap is very similar to that for the flat slide. However, before you start with the single cap calibration, you have to ensure that the capillary contact point on the sample slide has correctly been determined. Also, it is essential, that the plane tilt is defined for the single cap. If it is not the case, the capillary may break during one of the following 6 steps.

Procedure

1. Change to the objective with the smallest magnification and then move the XY-stage so that the cap is visible on the microscope, Fig. 6.34b. Then press **Next**.



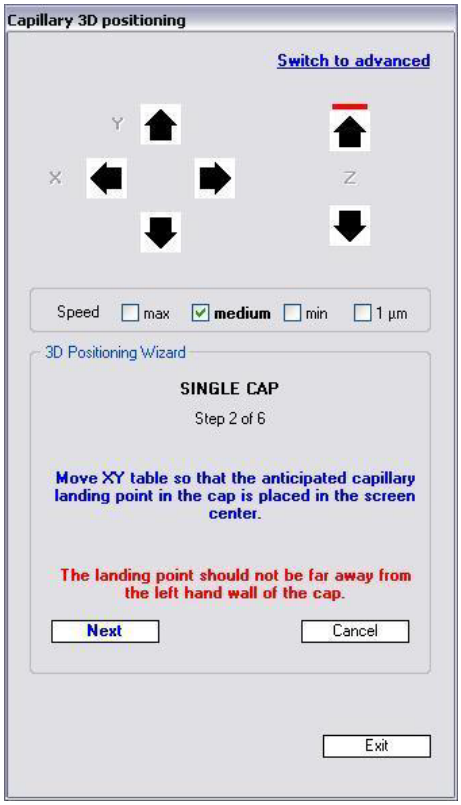
(a) The control panel.



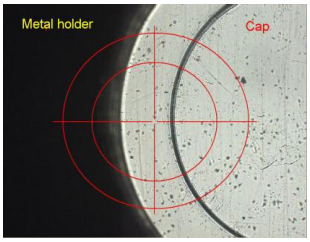
(b) The cap is visible on the microscope and centered to the marker.

Figure 6.34: *Step1 of capillary vertical positioning wizard with a cap.*

2. Move the XY-stage so that the future capillary landing point within the cap is placed in the screen center. This should be a location at the left hand side of the cap as shown in Fig. 6.35b. Then press **Next**.



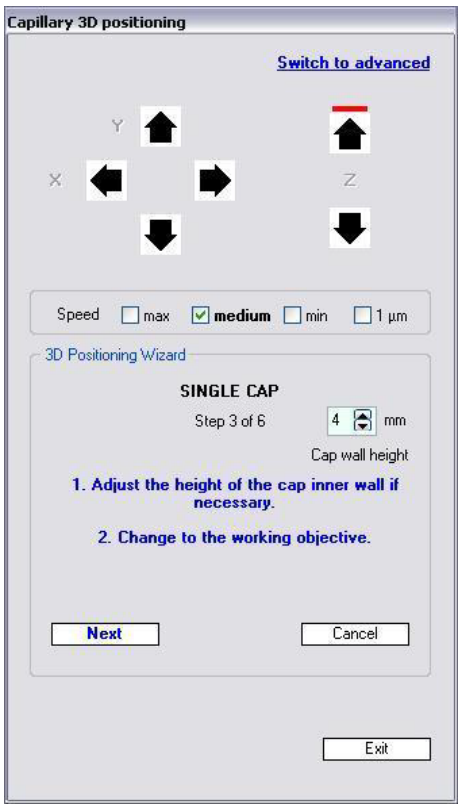
(a) The control panel.



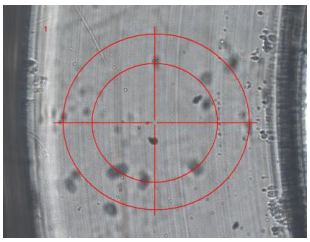
(b) The capillary landing point within the cap is placed in the screen center and located at the left hand side of the cap.

Figure 6.35: *Step2 of capillary vertical positioning wizard with a cap.*

3. If necessary, adjust the height of the inner wall of the cap. Then, change to the objective you anticipate to work with during collection of cells. Then press **Next**.



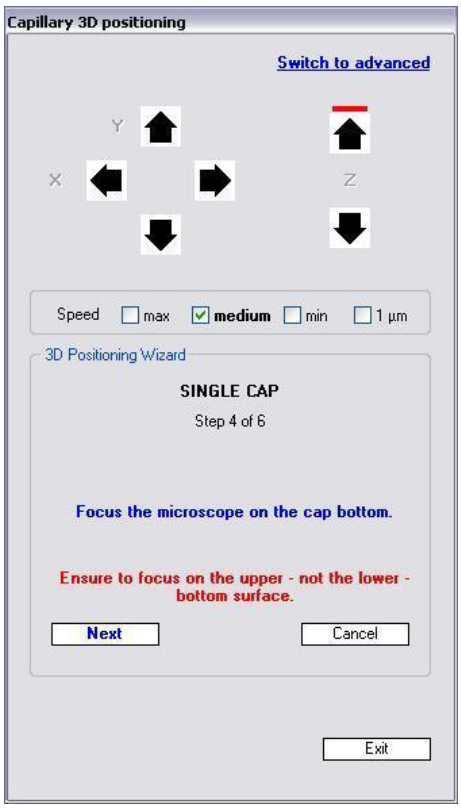
(a) The control panel.



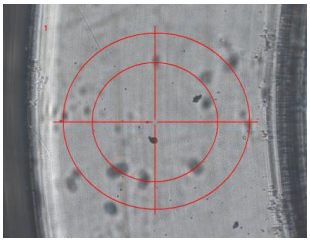
(b) Left side of the cap under high magnification working objective

Figure 6.36: *Step3 of capillary vertical positioning wizard with a cap.*

4. Focus the microscope on the cap bottom. Be sure to focus on the upper not on the lower surface of the cap bottom, otherwise the capillary may break in the next steps. Then press **Next**.



(a) The control panel.



(b) Left side of the cap under high magnification working objective focused on upper surface of teh cap bottom.

Figure 6.37: *Step4 of capillary vertical positioning wizard with a cap.*

5. The system moved the capillary to the position closely above the cap. The capillary is not seen under the microscope yet. At this moment, it is recommended to verify the position of the capillary by visual inspection of the XY-stage. The capillary body should be above the upper right edge of the cap in a distance exceeding the cap wall height; otherwise it would break in the next step. Then press **Next**.

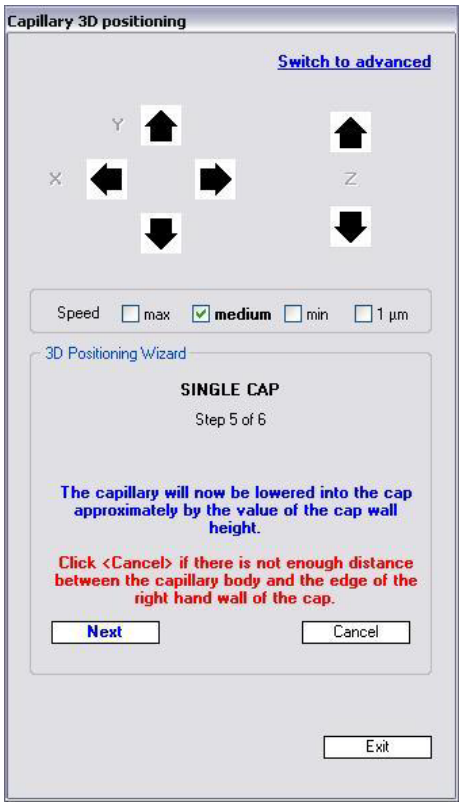
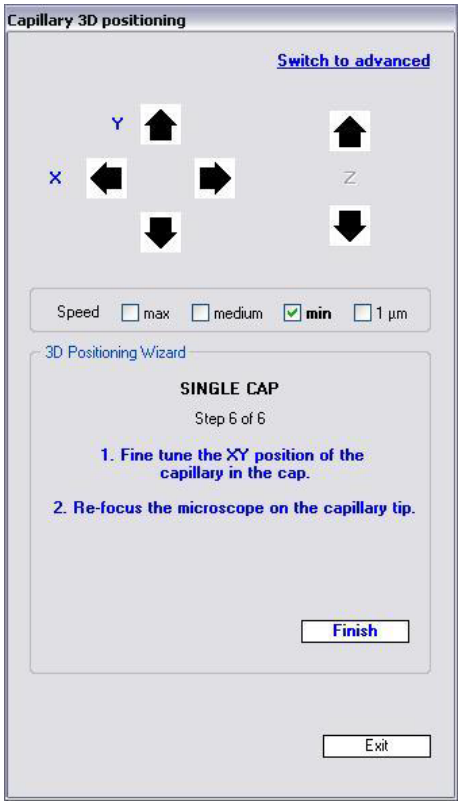
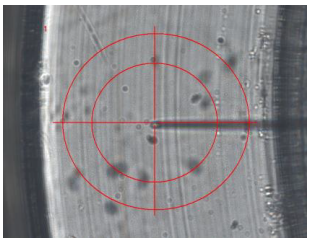


Figure 6.38: *Step5 of capillary vertical positioning wizard with a cap.*

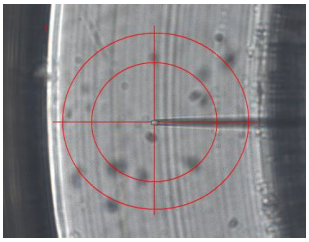
6. The system moved the capillary into the cap so that the capillary tip stays approx. 200 µmabove the cap bottom. The capillary tip may be out of focus at the moment, Fig. 6.39b. Now focus the microscope on the



(a) The control panel.



(b) Left side of the cap under high magnification working objective with unfocused capillary.



(c) Left side of the cap under high magnification working objective with focused capillary.

Figure 6.39: *Step6 of capillary vertical positioning wizard with a cap.*

capillary tip and fine-tune the XY position of the capillary if necessary, Fig. 6.39c.

> The capillary tip does not need to be placed in the screen center.

At the moment you press the **Finish** button, the system calculates the spacing between the capillary tip and the cap bottom (206 µmin the example shown

here). You can change this value later in the range from 0 (contact with the cap bottom) to 4000 µm.

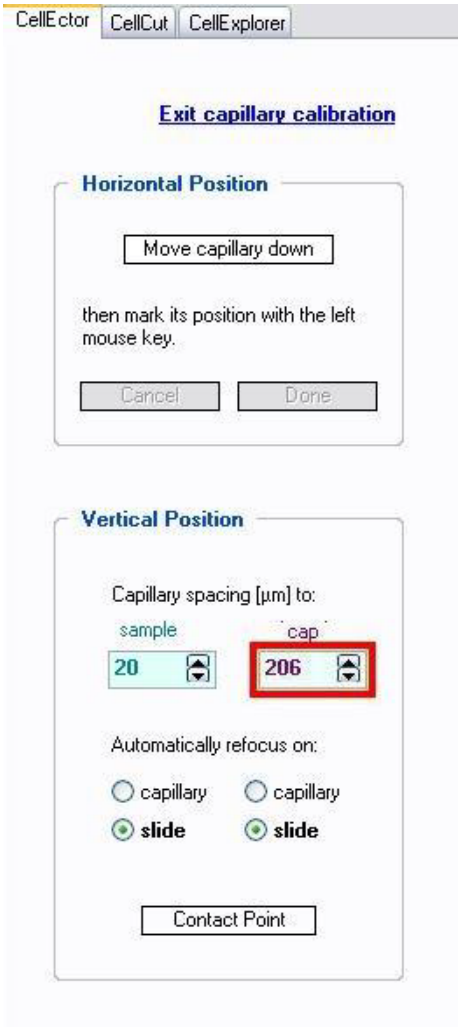


Figure 6.40: *System panel after finishing capillary vertical positioning wizard with a cap.*

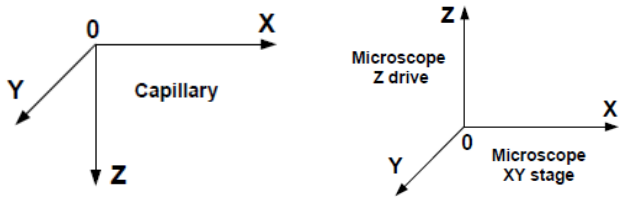
> **Large spacing values may conflict with the upper limit for the microscope Z drive if automatic refocusing on the capillary tip is selected or the user toggles the focus manually (using the Refocus on capillary/slide button in the action panel).**

The above calibration procedure sets not only the capillary position within the cap but also the cap position with respect to the XY coordinates of the screen. Thus, contrary to grids as deposit targets, there is no separate calibration procedure for the deposit position for the cap.

Capillary vertical contact position: advanced method In the advanced method, one has to be aware of two independent 3D coordinate systems used by *mmi CellEctor*:

- that of the capillary manipulator
- that of the microscope with its stage.

Their relative orientation is shown in the Fig. 6.41a and Fig. 6.41b. The XY



(a) 3d manipulator

(b) stage and microscope focus

Figure 6.41: *Coordinate systems used in mmi CellEctor.*

planes of the capillary and of the stage work fully independently. On the other hand, the relative position of the Z axes of the capillary and the microscope must be determined whenever a new capillary is inserted or its position is changed. For this purpose, the capillary is moved down to the contact position with the slide and the microscope is focused on both the capillary tip and the slide surface at the same time. At this moment the capillary Z position and the position of the Z drive of the microscope are stored for future calculations.

The controls of this advanced capillary 3D positioning dialog, Fig. 6.42 perform the following operations:

- **X**, **Y**, **Z** show the current position of the capillary. If you change any of these values using the spin controls, the changes are in effect after the **Go to** button has been clicked.
- **Go to** moves the capillary to the point defined by the current X, Y, Z values
- **Refresh** updates the X, Y, Z values
- **Move down** moves the capillary to the point defined as the contact point minus spacing. This button is invisible if the contact position is unknown.
- **Move up** moves the capillary to the point defined as the idle position (usually the uppermost one)
- **Joystick** activates/deactivates the joystick. Activation of the joystick disables the seven controls mentioned above. The position indicators X, Y, Z are, however, continually refreshed while the joystick is in use.

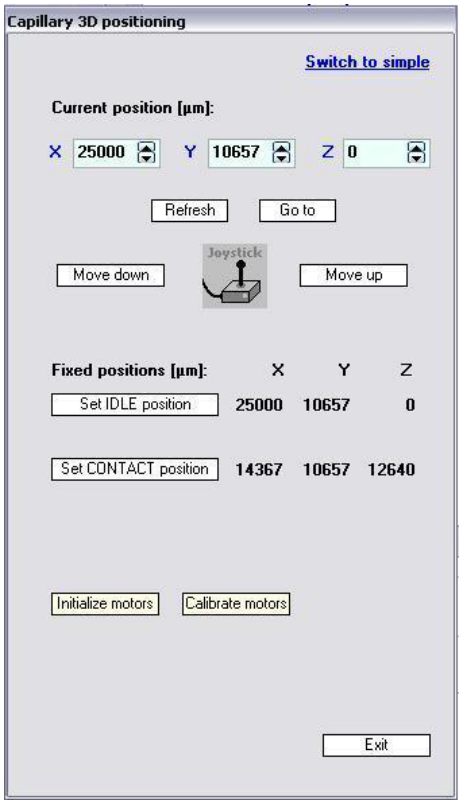


Figure 6.42: *Capillary vertical contact point calibration windows for the advanced method. Fixed positions only show up, if plane tilt is active.*

- **Set IDLE position** sets the idle position of the capillary
- **Set CONTACT position** sets the contact position of the capillary on a sample or a grid slide.
- **CAP position** is visible only for a single cap as deposit target and shows the current settings values for the capillary in the cap as determined in the simple method.
- **Calibrate motors** recalibrates the capillary motors X, Y and Z
- **Initialize motors** initializes the capillary motors X, Y and Z
- **Exit** closes the dialog and moves the capillary up.

Once the capillary orientation and the tilt have been selected, proceed as follows:

Procedure

1. Ensure that the Z drive control is ON (*Microscope →Z Drive Control* must be checked)

2. Activate the joystick.
3. Ensure that the capillary is high enough above the stage.
4. Move the capillary within the XY plane towards the optical axis of the microscope.
5. Lower the capillary down to approx. 2–3 mm above the slide plane.
6. Focus the microscope on the capillary tip either manually or by using the Z drive slider.
7. Center the capillary tip on the screen using the joystick.
8. Focus the microscope on the slide plane.
9. Using the joystick lower the capillary down to the slide until it touches the latter. The contact position can be recognized by the onset of the sideward movement of the capillary as shown in Fig. 6.43.

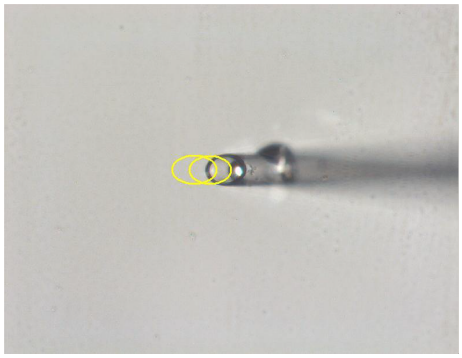


Figure 6.43: *Capillary starts moving sidewards, when touching the slide by moving the capillary down.*

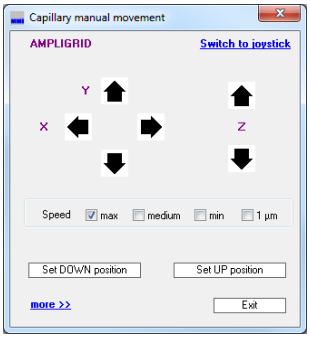
10. Click Set CONTACT position in the 3D positioning dialog. The current X, Y and Z values are stored as the coordinates for the down position of the capillary.

6.9.5 Capillary vertical contact position: manual microscope

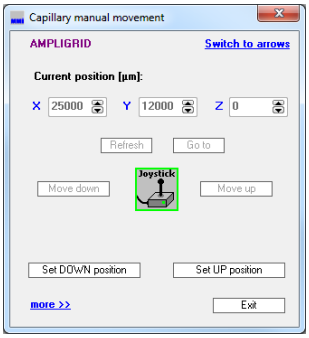
Defining the vertical reference position of the capillary at the contact with the slide is done in the 3D positioning dialog accessible through the link **Switch to system** or the menu

Cellector → *Capillary*

and than click on the button **Contact Point**. Calibration of the vertical contact position of the capillary for manual microscopes or if the plane tilt is not active is done in a single step. Use one of the dialogs Fig. 6.44a of Fig. 6.44b. The capillary has to be manually moved down to the contact position using either the XYZ arrows or the joystick. Once the contact position is reached, the button **Set DOWN position** has to be clicked. The idle (up) position is set by moving the capillary to this position and pressing **Set UP position**.



(a) using buttons



(b) using joystick and position numbers

Figure 6.44: *Capillary 3D positioning without active plane tilt support. Also used for manual microscopes.*

6.9.6 Capillary horizontal position on the screen

This calibration step is performed on the sample slide only.

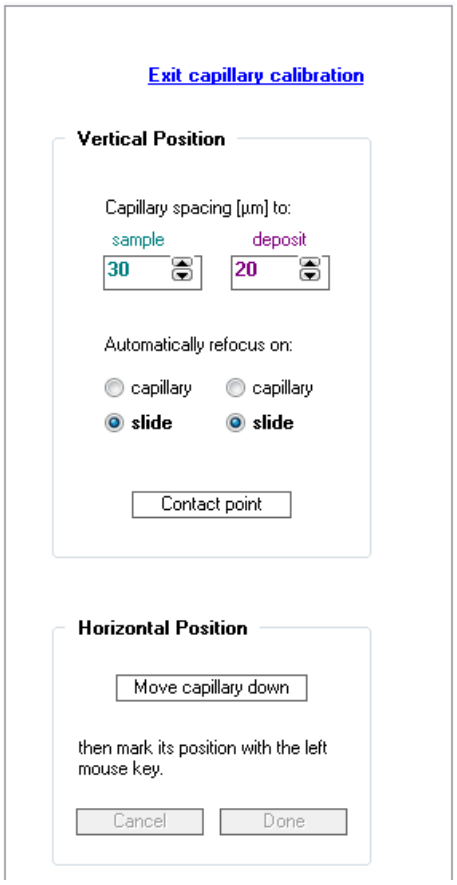


Figure 6.45: *Capillary horizontal contact point calibration window.*

Calibration of the position of the capillary tip on the screen is done in the panel shown in Fig. 6.45. Click **Move capillary down**, mark the tip on the screen with the mouse and click **Done**.

6.9.7 Deposit calibration

Calibration of the deposit is done in two different ways depending on the type of the deposit.

Calibration of grids Once the basic parameters of a grid (number of rows and columns, spacing between them) are known, it is enough to determine the location of the first spot (row 1 column 1, R1C1) on the XY-stage for the software to be able to calculate the locations of the remaining spots.

In the calibration routine

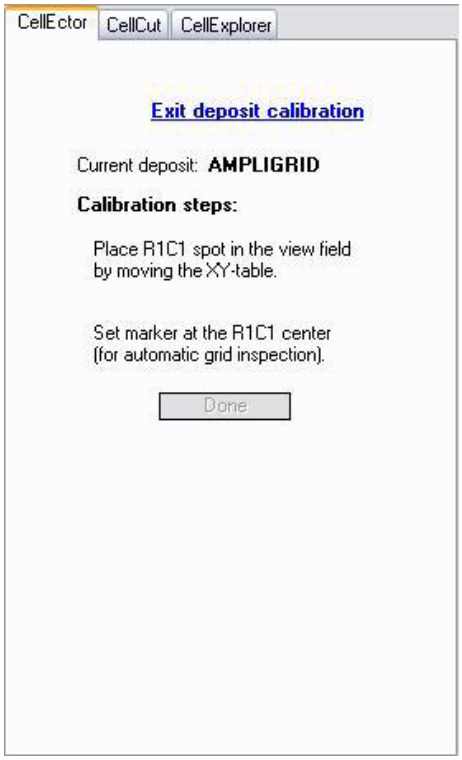


Figure 6.46: *Deposit calibration window.*

CellEctor $\rightarrow$ *Calibrate* $\rightarrow$ *Deposition*

or **Deposition** in the **system panel** the user has to set two markers at two locations at the R1C1 spot:

- the center of the spot
- the place where the capillary has to deposit the cell(s).

The first location is used by the software to center the image on the spot during the automatic grid inspection.

The second location is used for the deposition of cells. It may deviate from the spot's geometrical center in order to compensate for the asymmetry of the droplet formed during the pumping of liquid out of the capillary. **Calibration of a single cap** is made simultaneously with the calibration of the capillary position within the cap. Please refer to chapter 6.9.4 for the detailed description of the calibration procedure. The user is guided through the calibration with a self-explanatory wizard.

6.9.8 Service slide calibration

If one works with one of the MMI standard deposition slides (AmpliGrid or single cap) of known geometry, there is no need to calibrate the service slide. For non-standard deposition slides, a dedicated button **Service Slide** shows up in the system panel and opens a panel for a manual calibration procedure of the service slide.

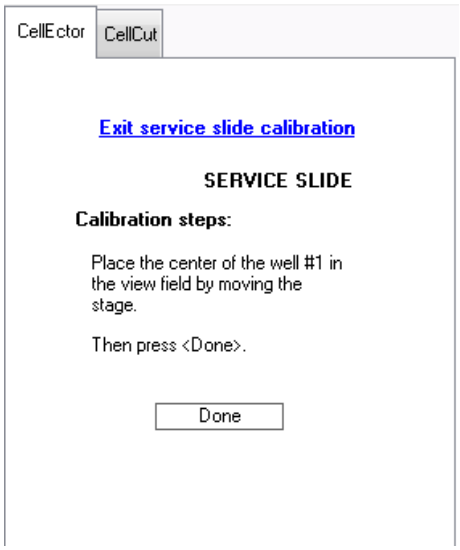


Figure 6.47: *Service slide calibration window.*

6.10 Using *mmi CellEctor*

mmi CellEctor can be used in a variety of modes starting with the manual mode, via the automatic mode ending with workflows running fully automatic after a single click.

In the manual mode, the basic steps of acquisition and deposition like moving the XY-stage, moving the capillary or pumping are not combined in sequence of actions. Thus, the user can perform a single step separately using the corresponding buttons in the action panel. Manual mode is used mainly for the tuning of the system to new experimental conditions (type of cells and buffer solution).

Once the optimum conditions for the movement of the capillary and pumping have been found, one of the buttons in either automatic or workflow mode can be used.

In the automatic mode, the work is divided in three steps: acquisition, deposition and rinse.

In the workflow mode, these three steps are combined in a series of actions (workflow). The simplest workflow is Acquire and deposit, the most advanced is Scan for cells. If required, the rinse step can be included in the workflows by checking the check box **With rinse**. A workflow can be interrupted by pressing *Esc*.

> While working in either automatic or workflow mode with the automated microscope, it is compulsory to determine the tilt of the plane both on the sample as well as on the target slide (except for a single cap).

6.10.1 Manual pump use

The pump is delivered ready for use, i.e. it is filled with oil and with the piston set in the backward position.

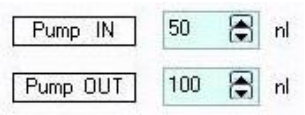


Figure 6.48: *Set the pump volumes in the action panel and initiate pumping by presseing **Pump IN** or **Pump OUT***

Pumping a predefined volume The buttons **Pump IN** and **Pump OUT** in the Action Panel allow pumping a pre-defined volume of liquid, see Fig. 6.48. The pump speed and acceleration can be configured as described in chapter

6.8.3. Once launched, the pumping cannot be interrupted. Thus, *mmi CellEctor* estimates the pumping time for a given volume and issues a warning if the time exceeds 2 minutes.

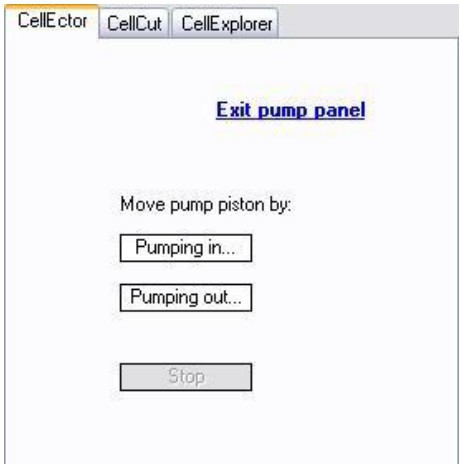


Figure 6.49: *Initiate continous pumping by presseing* ***Pumping in*** *or* ***Pumping out***

Endless pumping Continuous pumping with the highest speed and acceleration can be done using the pump panel accessible by pressing **Switch to system** →**Pump**, see Fig. 6.49. The pumping can be stopped by pressing the **Stop** button.

6.10.2 Moving the capillary up or down

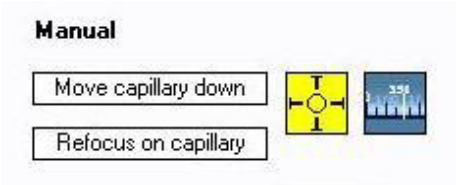


Figure 6.50: *Moving the capillary up or down by presseing* ***Move capillary up*** *or* ***Move capillary down***

Moving the capillary up or down is done by pressing the button **Move capillary up** or **Move capillary down**. The final down position of the capillary is calculated from the capillary contact position with the slide surface minus the spacing value set by the user in the action or in the capillary calibration panel. If the user changes the spacing value while the capillary is down it will be in effect yet in the subsequent down movement.

Button **Move capillary up** or **Move capillary down** are enabled only if both the upper (idle) and the lower positions of the capillary are known, i.e. the capillary is calibrated.

Whenever the capillary is down, the movement of the XY-stage is arrested except for the movement by dragging the mouse, if the user checked the latter option in the acquisition configuration dialog.

The software automatically recognizes if the capillary is up or down as long as the capillary movement is done solely by using the button **Move capillary up** or **Move capillary down**. The actual position of the capillary may mismatch the position concluded from this button if the user moved capillary with joystick. In such a case, repeatedly press the button **Move capillary up** or **Move capillary down** to remove the mismatch.

6.10.3 Automatic modes: Acquire, deposit and rinse

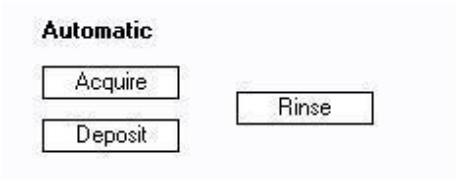


Figure 6.51: *Panel representing the automatic functions of mmi CellEctor*

In the automatic mode, the work is divided in three steps: acquisition, deposition and rinse. In order to separate a cell of interest from the sample and transfer it to the deposit, perform the following actions:

Procedure

1. Select a cell on the video screen by setting a marker
2. Click the **Acquire** button.
3. Once the selected cell has been aspired, click the **Deposit** button.
4. If required, click the button **Rinse**. The capillary moves up, the stage moves to the to service slide and the capillary is rinsed.
5. Open the context menu by clicking on the video image with the right mouse button and select **Back to sample**. The stage moves back to the point on the sample, where the cell has been aspired.

If the cell could not be aspired in step 2, the user has the possibility to perform additional pumping step(s) by pressing button **Pump** in in the manual part of the action panel (provided that the option **Leave capillary down** after acquisition has been checked in the acquisition configuration dialog).

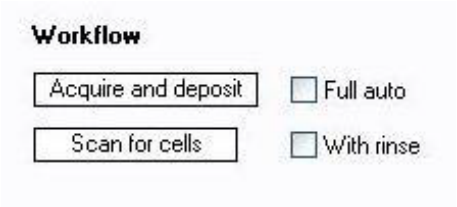


Figure 6.52: *Panel representing the workflow functions of mmi CellEctor*

6.10.4 Acquire and deposit workflow

To acquire a cell from the sample slide and deposit it automatically to the deposit slide mark a cell and click the button **Acquire and deposit**.

If the check box Full auto is checked, acquisition and deposition of the cell is launched immediately after a marker is set. Thus, there is no opportunity to delete a marker that has erroneously been set on a wrong cell.

6.10.5 Scan for cells workflow

With the **Scan for cells** workflow, the user does not need to mark the cells since it is done automatically be the software. This workflow is usually applied under fluorescence conditions since the colored cells of interest can easily be distinguished from the black background.

A single click on the **Scan for cells** button in the action panel launches the following recurrent workflow:

Procedure

1. A snapshot of the video screen containing the cells is made at the starting position of the stage and is then transferred to the image processing software *mmi CellExplorer*.
2. *mmi CellExplorer* finds the cells, marks them and sends the set of the markers back to *mmi CellEctor*.
3. *mmi CellEctor* selects a single cell to be aspired.
4. acquisition
5. deposition
6. rinsing (if required)
7. moving back to the sample

8. Depending on the settings made by the user, either another snapshot is made at the same stage location, or the stage is moved to the next location and the loop restarts.

In order to scan through the sample, the user can define the number of screens horizontally and vertically in the acquisition configuration dialog, see chapter 6.8.4. Since usually there are several cells of interest within one snapshot, the recognition and selection of cells can be repeated several times at the same stage location.

> For the fully automatic scanning to work correctly, it is compulsory to load the correct set of parameters to *mmi CellExplorer* software before you start the scan. Please refer to the *mmi CellExplorer* manual for the explanation how to do it.

6.10.6 Automatic inspection and reporting of deposit grids

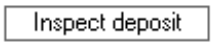


Figure 6.53: *Inspect deposit button*

By pressing **Inspect deposit** *mmi CellEctor* is capable to automatically scan through the spots of a grid giving the user opportunity to verify the presence of cells in the spots. The verification may be either just visual or using automatic cells recognition by *mmi CellExplorer*. The inspection process can be configured as described in chapter 6.8.7.

Reports If the reporting is actived in the confiration dialog, see chapter 6.8.7, a snapshot of each inspected spot is stored in the JPG format for documentation purposes. The image files are named RxCy.jpg where R, C represent row and column, and x, y represent their number, respectively. The images are stored it the folder

```
My Documents\mmiCellTools\AutoDoc\
\CL_InspectData_yyyy-mm-dd_hh-mm-ss\RxCy.jpg
```

Furthermore, a report file can be created in the ASCII tab separated format that contains the following information:

- Spot ID number (RxCy).
- Number of cells automatically recognized in each spot during the inspection of the grid.
- Total amount of liquid pumped out to each spot.

The file can be generated from the main menu item

CellEctor → *Export Grid Data*

6.11 Optimization of the system for collecting cells

There are two key steps in the process of collecting rare cells:

- getting a cell into the capillary at the sample
- getting the cell out of it at the target

Since there is a large variety of cells and of buffer solutions, the working parameters of *mmi CellEctor* have to be optimized for the given experimental conditions. The major optimization parameters are:

- pump parameters
- spacing between the capillary and the slide surface
- size, tilt and orientation of the capillary

Please note, that prior to the tuning of the system, one has to determine the tilt of the slide plane(s).

6.11.1 Pump parameters

In order to understand the meaning of the pump parameters, let us go through an example of the following series of the user's actions:

Procedure

1. Acquisition of a cell.
2. Deposition of the cell.
3. Acquisition of another cell, however, the cell could not be pumped in the first attempt.
4. Retrial to pump the cell in.

The Fig. 6.54 shows how the pump behaves during these four actions under assumption that the following pump parameters have been set:

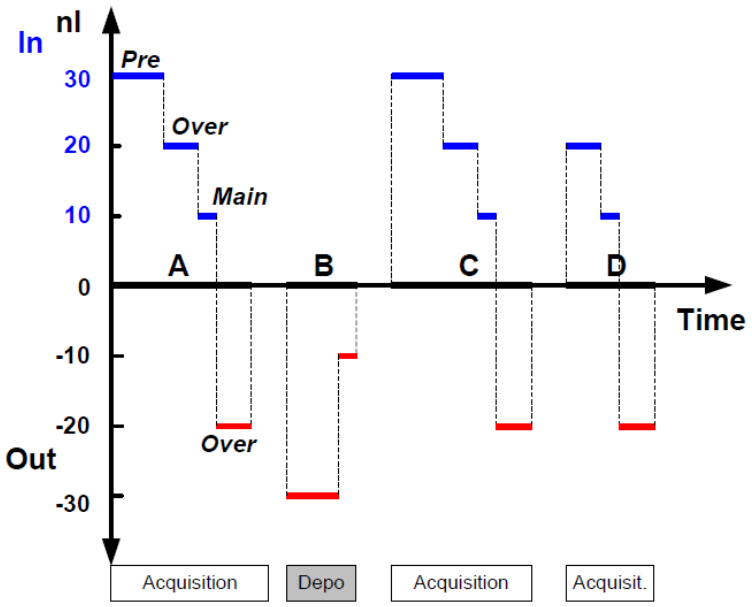


Figure 6.54: *Panel representing the workflow functions of mmi CellEctor*

- Main volume 10 nl for both in and out
- Pre-pumping volume 30 nl
- Over-pumping volume 20 nl

During the acquisition (1.) of the cell, 30 nl (pre-pumping) are pumped in at first. We assume here that pumping out took place just prior to it, thus the pump changes the pumping direction and 30 nl are necessary for the compensation of the overpressure remaining after pumping out. In the second step, 20 nl are pumped in (over-pumping) that will be then pumped out in the fourth step. Thus, over-pumping does not add net volume to the main volume. It helps, however, to pump in cells that adhere to the slide surface. In the third step, the main volume of 10 nl is pumped in. Thus, totally 40 nl is pumped in during the acquisition process.

During the subsequent deposition (2.) of the cell, 30 nl are pumped out at first (pumping direction again changed) and then 10 nl of the main volume, totally 40 nl. The next acquisition action (C) proceeds exactly the same way as the first one (A). However, the user launches an additional pumping-in action (D) in order to get the cell into the capillary. Since the pump did not change its direction (the over-pumping volume that is pumped out at the end of the pumping in does not count), no pre-pumping volume is added to the main volume this time.

There are no universal rules for choosing the values of the pump settings, however, several practical hints can be given:

- The smaller is the diameter of the capillary tip, the slower is the dynamics of the fluid movement through the tip and the larger is the under/over-pressure necessary to pump in/out. If the dynamics is slow, one has to increase the pre-pumping volume, the relaxation time, as well as the speed and the acceleration of the pump.
- Light cells can be moved into the capillary with a small main volume and no over-pumping.
- Heavy cell objects, e.g. cells that are labeled with a number of paramagnetic beads, tend to quickly penetrate the capillary even at small main volume values. In order to catch such cells, reduce the main pump-in volume to nearly zero and work with over-pumping volume solely. The pump speed and acceleration should also be reduced in this case.
- Whenever possible, use capillaries with a large tip diameter, even if the cells are relatively small. The optimum pump parameters can be much easier found for such capillaries and the pumping process is faster.

6.11.2 Spacing

The distance between the capillary tip and the surface of the slide affects the efficacy of the cell acquisition and deposition in a quite significant way. There are different practical rules of thumb for setting the spacing value depending on the orientation of the capillary bevel with respect to the slide surface:

6.11.3 Capillary bevel parallel to the slide surface

If the capillary bevel is oriented parallel to the slide surface, the cells get into the capillary by the vertical flow forces. Following rules apply:

- The smaller the spacing on the sample, the lower the amount of the liquid that has to be pumped in to get a cell into the capillary. One has, however, to take into account the size of the cells as compared to the diameter of the capillary tip and the height of the cells.
- The above rule does not apply for pumping the cells out on the deposit. If the deposition takes place on a dry surface, the surface tension of the liquid has to be taken into account which, at small spacing, may prevent the liquid from flowing out of the capillary.
- The optimum spacing on the deposit depends on the amount of liquid to be pumped out and vice versa. Both parameters should be chosen so that the liquid touches the deposit surface and flows out of the capillary before a droplet is formed and falls down.

- If the deposition takes place into a single cap, spacing should in principle be set to zero. Nonzero values may still be used to correct for the liquid boundary moving upon evaporation in order to keep the capillary tip within the liquid.

6.11.4 Capillary bevel vertical to the slide surface and cell scratching

If the capillary bevel is oriented vertically to the slide surface, the flow forces (parallel to the slide surface) can be combined with a scratching action of the capillary. Scratching is achieved by sliding the capillary tip along the slide surface. The capillary will move in this way if the spacing is set to a negative value since the capillary narrow end part will be deflected by the slide surface. However, be aware, that the capillary may break if the negative value exceeds -10 µm.

> Please observe the safety precautions described in the first chapter of this manual. Capillary size, tilt and orientation. Some hints concerning the size have already been mentioned above. The tilt and the orientation can be arbitrary except for the case when the tip bevel should be parallel to the slide surface and the tilt should be equal to the bevel angle.

6.11.5 Collecting cells to a matrix of spots (grid)

Since there is a variety of commercially available grids, we will describe collecting of cells to AmpliGrid as an example. Most of the steps apply, however, to all other grids as well.

If a new grid is placed under the microscope, the following operations have to be performed in order to make the grid ready for cells:

Procedure

1. Notify the software about the new grid: Open the **deposit configuration** dialog, select the type of the grid, set its parameters if necessary (custom grid) and check the option **New grid inserted**, if it has not automaticallybeen checked.

   > This deletes all the data stored for the previous grid.

2. Set the number of cells you want to deposit at each spot. Please note that this number is used to determine whether or not the spot is full, i.e. whether the next cell can be added to the current spot. Close the deposition configuration dialog.

3. Determine the tilt of the grid plane.
4. Optionally, determine the contact point of the capillary on the new grid. This is necessary only if the thickness of the new grid significantly differs from the previous one and/or a high accuracy of the spacing between the capillary and the grid is required.
5. Calibrate the position of the spot located in the first row, first column (R1C1).
6. Perform the first deposition at the first spot of choice leaving the capillary down after pumping out. If the buffer solution with a cell could not leave the capillary after having pumped out the preset volume, increase the latter or decrease the capillary spacing to the deposit, whatever appropriate.

Continue with filling all other spots with cells. The software registers the number of deposition actions in each spot and assumes that one cell is deposited each time. Once every spot in the grid is filled with the preset number of cells, the entire grid is full, no further deposition is possible and the user is prompted to replace the grid.

> One can also fill a selected part or every second spot on the grid only. To do the former, simply define the current and the end row/column in the deposit configuration dialog. To do the latter, define a custom grid with spacing between the columns that is double the spacing and the number of columns (rows) that is a half of the number of columns (rows) on the actual grid.

6.11.6 Standard settings for beads

The settings in the Table 6.1 apply to 10 µmpolystyrene beads in aqueous solution. Concentration of the beads should be chosen so that approximately 20 beads are seen at once in the field of view with a 20X objective.

6.11.7 Collecting cells to a single cap

To collect cells to a single cap, one works with a transparent cap placed in a cap holder on the XY-stage. Assuming, that all the operations necessary to optimize the acquisition process on the sample have already been done, one has to calibrate the position of the cap and that of the capillary in the cap.

Once the cap position has been determined, one can proceed with the acquisition and the deposition of cells using one of the automatic modes.

Table 6.1: *Standard settings for beads*

Capillary	
Bevel orientation	Parallel to slide
Tip diameter	40 µm
Spacing to sample slide	5 - 10 µm
Spacing to AmpliGrid	50 µm

Pump	
Pump in volume	10 nl
Pump out volume	100 nl
Pre-pump volume	0 nl
Over-pump volume	0 nl
Relaxation time	0 ms
Speed	100%
Acceleration	100%

7 Automated microscope control

mmi CellTools supports the following automated microscope types:

- Olympus IX81
- Nikon Eclipse Ti
- Nikon TE2000 E

The automated microscope control functionality must be installed by an authorized MMI technician.

7.1 Activating microscope control

To enable automated microscope control, the microscope's controller hub must be connected to the PC (either COM port or USB). To make *mmi CellTools* connect to the microscope, activate the menu item

Microscope → *Remote control*

This activates control of objectives, filter block, condenser cassette, fluorescence shutter and lamp brightness. The focus knobs on the microscope body will still be useable.

mmi CellTools will remember these settings and restore them at startup. Make sure the microscope is connected and switched on before starting *mmi CellTools*.

7.2 Observation methods

The setup editor is used to define the observation methods, see section 5.2.2.

In the setup editor you define for each setup the position of the

Table 7.1: *Optical path settings for Olympus and Nikon microscopes*

	Olympus	Nikon
Left side port	1	5
Binocular	2	1

- Filter block
- Condenser
- Optical path (Camera port, Binocular)

When you change the setup, the motorized microscope will set these three items to the positions defined in the new setup automatically. The optical path settings are specific to microscope vendors (see Table 7.1).

Filter wheel position numbers and condenser turret position numbers are clearly indicated on the microscopes.

A fast switch from any camera port to binocular and backwards is performed in the menu

Microscope $\rightarrow$ *Binocular*

or by pressing *F7*.

7.3 Objective control

In the objective editor, see section 5.2.3, you can save nosepiece position number and the lamp voltage used.

When changing the objective the corresponding nosepiece position and lamp voltage will be established automatically by the microscope. Nosepiece position numbers are clearly indicated on the microscopes.

The lamp voltage setting may be different for different objectives. Therefore it is recommended to enable the per-objective camera white balance setting (see section 5.3.2).

7.4 Z drive control

CellTools features built-in focusing aids and functions that rely on motorized z drive control. These are:

- Sample plane definition
- Focus memory for pin positions

mmi CellTools offers three ways to manually focus the z drive:

1. Using the vertical slider on the right-hand side for coarse focussing;
2. Using the two arrow buttons at each end of the slider for fine focusing and
3. Using the mouse wheel.

In order to use the mouse wheel for focusing, click the **mouse wheel focus** button. While the button is activated, the mouse wheel is linked to the microscope z-drive.

Of course, it is possible to refocus using the microscope's built-in focusing controls.

> When using the manual focus wheel on the microscope, some microscope models may not feedback the changed focus values into the software. In order to notify CellTools that the focus has changed, click on the numerical focus display below the slider to update its value.

7.4.1 Required calibrations

To estimate the correct focus position requires:

- Correctly measured location of the membrane slide in three dimensions → Sample plane definition
- Correctly measured z-drive offsets for
  - different objectives

7.4.2 Sample plane definition

The sample plane focus mechanism is a simple focussing aid based on the assumption that the sample is planar, but slightly tilted against the horizontal plane of the stage. As a consequence, the focus always lies on this plane, which can be defined by three points on the slide (see Fig. 7.1).

When moving to a different location on the slide, the focus is automatically adjusted according to the sample plane definition.

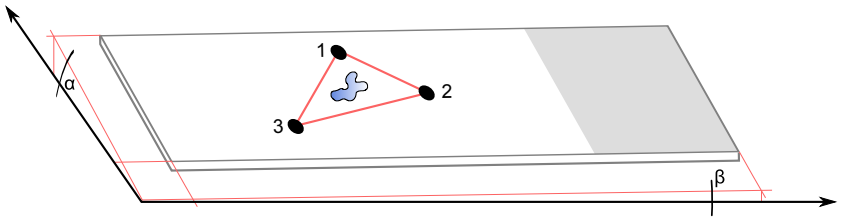


Figure 7.1: *Definition of a tilted sample plane. Given three arbitrary base points (1, 2 and 3), the software computes the tilt angles α and β with respect to the horizontal plane.*

> While plane tilt focussing is a useful focussing aid on glass slides and for small areas of interest, the approach is limited. By principle, this method cannot compensate for rugged and non-planar surfaces. For such samples, it is recommended to switch plane tilt focussing off (see below).

To properly define a plane in three dimensions, three points must be set. These points should not be on a line. To get most precise results use a high magnification objective for this procedure (20x or higher).

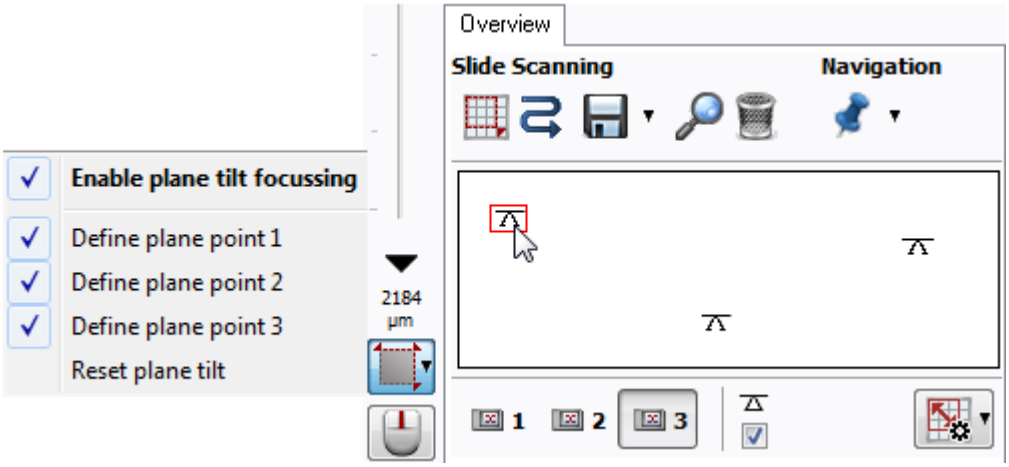


Figure 7.2: *Plane tilt user interface*

Procedure

1. Move to a location near the edge of your sample and focus the microscope.
2. Select *Define plane point 1* from the plane tilt menu (Fig. 7.2) or press *Ctrl + 1*.
3. Move to a second suitable location and focus.
4. Select *Define plane point 2* or press *Ctrl + 2*.

5. Move to a third suitable location and focus.
6. Select *Define plane point 3* or press *Ctrl + 3*.
7. Repeat this procedure for any other slide on the tray.

Note that the positions of the plane points are displayed in the overview scan.

After the plane is defined, the sample plane focussing aid can be switched on and off through the plane tilt button or menu (*Enable plane tilt focussing*). Any of the three points can be redefined individually afterwards to refine the plane definition.

> The orientation of the sample plane is stored independently for each slide. When changing to a slide, for which no sample plane has been defined, plane tilt focussing will be disabled.

You may use *Reset plane tilt* to remove all points and start defining a plane from scratch.

> Neither the single cap nor the service slide require the determination of the plane tilt.

7.4.3 Automatic Z drive control

Each time you move the stage with the arrow keys or mouse the best Z drive position will be calculated and the Z drive motor moved to that position. With flat samples you immediately receive a sharp image. The more uneven the sample is, the more you need to correct the Z position manually.

If you draw a shape or if you set a pin position the software saves the z position. Each time you go back to the shape or the pin position, the saved Z position will be recalled. You never need to refocus during cutting.

7.4.4 Z drive offset for slide

In the case of a slide which is non-planar, it is necessary to define a Z drive offset for the current area. This distance can be measured and corrected automatically.

Procedure

1. Focus microscope

2. Select menu item

   Microscope →Z Drive Offset →Slide

   or press *F8*.

After successful calibration the scope does not loose the focus, when you change the position of the stage slightly.

7.5 Microscope safety parameters

mmi CellTools provides functions to help prevent physical damage to the microscope objectives. These functions are always in place and do not require manual intervention. However, under certain circumstances, they can be configured through the safety parameters dialog.

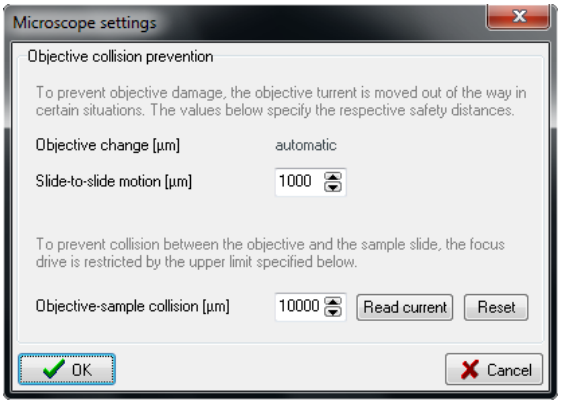

Microscope settings
Objective collision prevention
To prevent objective damage, the objective turrent is moved out of the way in certain situations. The values below specify the respective safety distances.
Objective change [µm]
automatic
Slide-to-slide motion [µm]
1000
To prevent collision between the objective and the sample slide, the focus drive is restricted by the upper limit specified below.
Objective-sample collision [µm]
10000
Read current
Reset
OK
Cancel

Figure 7.3: *Microscope safety parameters*

Open the dialog using the menu item

Microscope →Safety parameters. . .

7.5.1 Objective collision prevention

There are two situations in which mmi CellTools automatically lowers the objective turret to prevent potential damage to the objectives:

The first situation is when switching to another objective. This function is carried out automatically and does not require configuration.

The second situation is when moving the stage to a different slide. If the two slides are not perfectly aligned the surface of the objective may scratch against the slide's lower surface. To reduce the risk of damage, the objective turret is

lowered by a certain amount, which can be specified in the safety parameters dialog (Slide-to-slide motion). If the slides used in your setup exhibit very high tolerances you may wish to increase this value.

When you are using the small step arrow keys or the mouse the z drive escape function is switched off.

7.5.2 Objective-sample collision

To prevent contact between the objectives and the specimen and to protect the microscope itself, the range of the focus drive is automatically limited to a certain maximum value, which can be specified in the safety parameters dialog.

To use the current focus setting as objective-sample collision limit, press the Read current button. To reset the limit to its default value, press Reset.

The recommended procedure for setting the objective-sample collision limit is:

Procedure

1. Select the objective with the shortest working distance (normally the objective with the highest magnification)
2. Focus the microscope
3. Open the safety parameters dialog:

   Microscope $\rightarrow$ *Safety parameters...*
4. Press the **Read current** button
5. Add the working distance in µm to the displayed value and go to step 8, or if the working distance is unknown proceed with step 6.
6. Carefully move the objective as close as possible to the specimen without touching it.
7. Write down the value displayed under Objective-sample collision.
8. Press the Read current button again.
9. Now enter an Objective-sample collision limit half-way between the two positions.
10. Click OK

7.6 Fluorescence shutter control

If installed, the microscope's epifluorescence shutter can be opened and closed via software. Select

Microscope → *Shutter*

or press *F6*.

8 Image analysis using mmi CellExplorer

mmi CellExplorer is an optional image analysis software module for *mmi CellTools*. It was developed to find, count and measure microscopic objects for subsequent extraction or manipulation. A carefully selected and application-adapted set of functionalities guarantees a high throughput at a comfortable level of automation. Nevertheless, a high degree of flexibility is supported.

The *mmi CellExplorer* finds, counts and sorts objects primarily based on the object colors. Secondly, the automatic distinction of various object shapes is a particularly innovative feature. Round shapes (undifferentiated cells), for instance, can be automatically distinguished from oval forms (differentiated cells). Objects adjacent to each other are automatically separated and counted by the software. Finally, the object shapes found can automatically be optimized for laser cutting.

For advanced users the *mmi CellExplorer* comprises complex image preprocessing that can be performed before the object finding. For this purpose the software has many different filters like image sharpness, color intensity, brightness, color space transformations, background separation by fast Fourier transformations, gamma corrections.

8.1 Getting started

Switch from *mmi CellCut* or *mmi CellEctor* to the *mmi CellExplorer* by choosing the *mmi CellExplorer* tab. The live image is loaded to the *mmi CellExplorer*.

8.1.1 Encircling objects of one uniform color

By clicking on the plus left to the **color** label the small color panel (Fig. 8.1) opens.

This panel shows eight fields representing eight different object types you can search for.

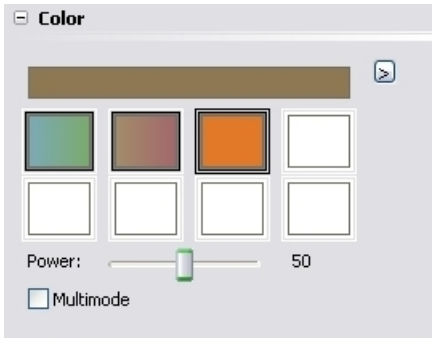


Figure 8.1: *Color panel*

Move the mouse cursor onto the displayed image and left-click on the color of the object you would like to count. The color will be displayed on the left side of the first of the eight color fields in the settings panel.

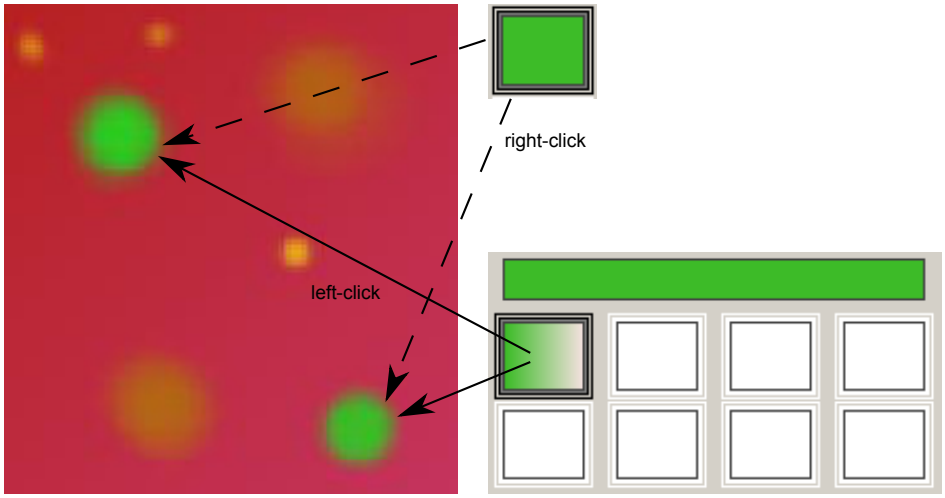


Figure 8.2: *Detecting objects of uniform color*

With a **left mouse click**, the color under the cursor will be entered into the left side of the first color field. The computer would search for all objects with color ranging from green to white. The gradient in this color field displays the "search color".

A **right mouse click** will choose the "end-color" of the color gradient to search for. In our case of a unicolored object select the same object with a left and the right mouse click. The color field will now be filled with green only. Only green objects will be searched for.

A **left and right mouse click** onto a unicolored object searches unicolored objects with the same color (Fig. 8.3).

If the objects are small, it can be tricky to hit the right color. By using the zoom options of the *mmi CellExplorer* (section 8.2.2) the selection will be very easy and precise.

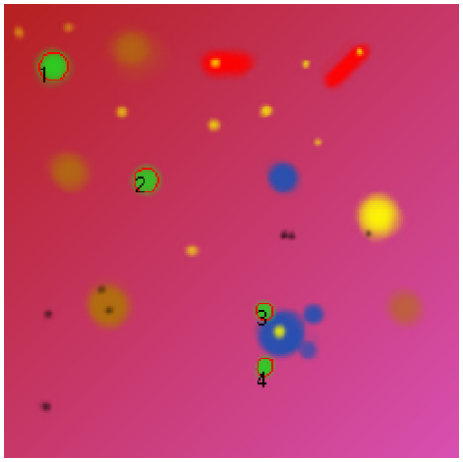


Figure 8.3: *Detecting objects of uniform color: Resulting objects*

8.1.2 Encircling Objects with different uniform colors

In order to search for objects with different colors you need more than one color field. Corresponding to the eight color fields, you can define up to eight object types for your search. In order to define the colors for those objects activate the respective color field:

Activate the field for color editing with a left-click. Only one color field can be edited at once. The outer frame of the color field indicates the selection status (black for active, light gray for inactive)

Activate/deactivate the field for object search with a right-click. The inner frame of the color field indicates the status (see section 8.2.3.4).

You can find, count and calculate objects with different colors separately with the *mmi CellExplorer* software (see Fig. 8.4).

8.1.3 Encircling Objects with color gradients

Many objects show a color gradient. The *mmi CellExplorer* can detect them as well as uniform colored objects. Select the start color of your gradient with a left-click and the end color with a right-click. Differing brightness or intensity of the same color can form the gradient too.

Note: Only activated color fields will be used for object search.

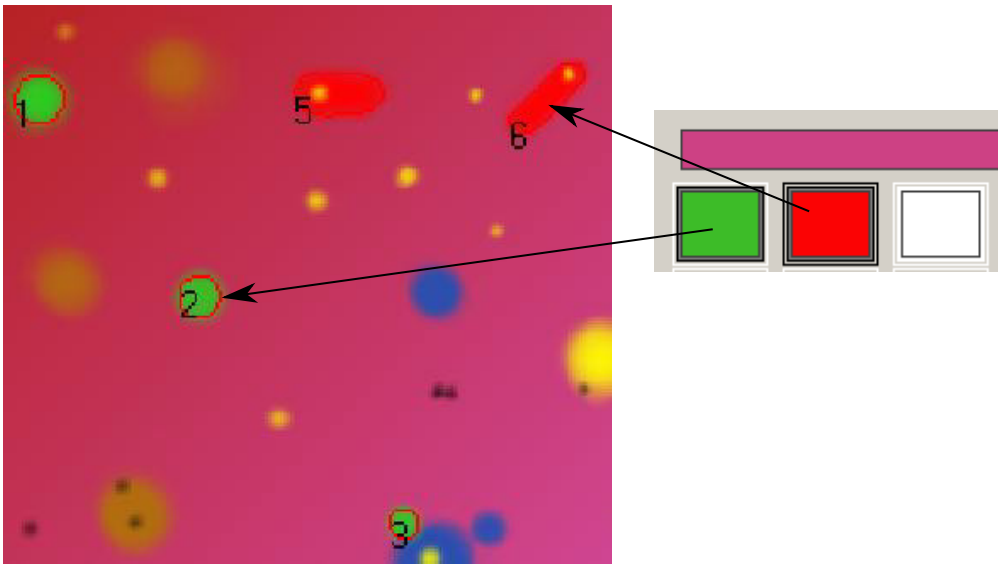
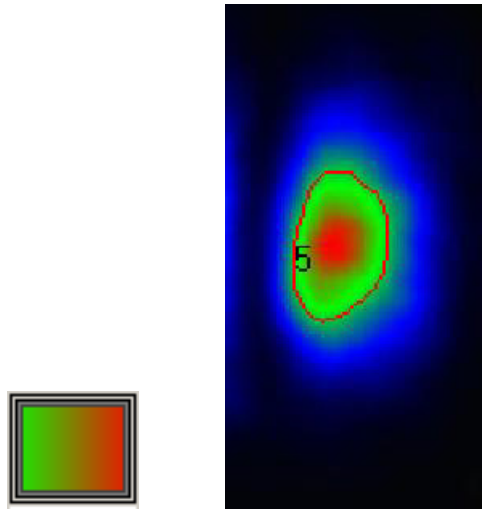


Figure 8.4: *Two colors selected*



(a) Color field (b) Result

Figure 8.5: *Green was selected with the left mouse click, red with the right mouse click. The selection is displayed in the color field as a gradient. All colors contained in the gradient will be searched for during a calculation.*

8.1.4 Transfer to other mmi CellTools modules

After selecting your colors, click on the calculate button (green arrow) in the process toolbar. The software will now search and mark your objects. Please wait until the process has been completed. This can take a while for large images.

To return to the main application, click the **Return to application** button at the top of the *CellExplorer* panel. After switching back all detected cells are lined out and stored in the group statistics. Now you can start the cutting or collecting process of all marked specimens.

8.1.5 Optimization of results

If your result is not totally satisfactory you can optimize each step of the object detection. Please refer to section 8.2.

The settings for optimizing the object detection procedure described herein can be executed directly in the respective section of the Settings panel.

8.1.6 Result lists

8.1.6.1 Object list

The following information is available via the menu item

View $\rightarrow$ *Object list*

after each object detection. It displays the calculated parameters like diameter, total area, circumference and roundness for each detected object.

8.1.6.2 Count list

Under the menu

View $\rightarrow$ *Count list*

the count list shows a summary of the detection results. The table lists the number of objects as well as relative area, sample information and respective calculated objects per unit in your sample.

	ImageNo	ObjectNo	ColorNo	Unit	Diameter	TotalArea	Circumference	Roundness	RelativeArea %	DateTime	ImageFile
	1	1	1	µm	120.5660	10306.7551	409.4428	1.1077	6.1333	9/17/2009 1:35:52 PM	Img___1
	1	2	1	µm	106.9294	7978.2835	350.6021	1.1256	4.7477	9/17/2009 1:35:52 PM	Img___1
	1	3	1	µm	95.8472	6349.4134	305.8837	1.1364	3.7784	9/17/2009 1:35:52 PM	Img___1
	1	4	1	µm	158.0753	14469.0305	474.1817	1.3564	8.6102	9/17/2009 1:35:52 PM	Img___1
	1	5	1	µm	80.8497	4353.0759	253.4891	1.1794	2.5904	9/17/2009 1:35:52 PM	Img___1
	1	6	1	µm	124.2890	8529.4847	370.8822	1.4224	5.0757	9/17/2009 1:35:52 PM	Img___1
	1	7	1	µm	141.2918	11550.4910	414.7095	1.3574	6.8734	9/17/2009 1:35:52 PM	Img___1
	1	8	1	µm	67.9304	3141.1400	208.7576	1.1538	1.8692	9/17/2009 1:35:52 PM	Img___1
	1	9	1	µm	105.5324	6529.6138	311.5219	1.3396	3.8856	9/17/2009 1:35:52 PM	Img___1
	1	10	1	µm	94.0611	4886.6103	267.0753	1.4220	2.9079	9/17/2009 1:35:52 PM	Img___1
	1	11	1	µm	71.5282	2982.1396	206.1013	1.3475	1.7746	9/17/2009 1:35:52 PM	Img___1
	1	12	1	µm	72.5581	3077.5398	222.2597	1.3436	1.8314	9/17/2009 1:35:52 PM	Img___1
	1	13	1	µm	73.8612	3063.4065	222.3631	1.3987	1.8230	9/17/2009 1:35:52 PM	Img___1
	1	14	1	µm	93.1931	4964.3438	294.6811	1.3740	2.9542	9/17/2009 1:35:52 PM	Img___1
	1	15	1	µm	94.3985	4890.1437	286.6229	1.4312	2.9100	9/17/2009 1:35:52 PM	Img___1
	1	16	1	µm	108.5039	6815.8144	345.5217	1.3566	4.0559	9/17/2009 1:35:52 PM	Img___1
	1	17	1	µm	127.6272	9317.4197	391.4636	1.3730	5.5446	9/17/2009 1:35:52 PM	Img___1
	1	18	1	µm	106.3661	6681.5474	319.9865	1.3299	3.9760	9/17/2009 1:35:52 PM	Img___1
	1	19	1	µm	139.5050	11875.5584	434.9877	1.2871	7.0669	9/17/2009 1:35:52 PM	Img___1
	1	20	1	µm	159.2555	14631.5642	497.4977	1.3614	8.7069	9/17/2009 1:35:52 PM	Img___1
	1	21	1	µm	125.0543	9356.2864	384.0695	1.3128	5.5677	9/17/2009 1:35:52 PM	Img___1
	1	22	1	µm	146.3044	12296.0259	435.5593	1.3672	7.3171	9/17/2009 1:35:52 PM	Img___1

Figure 8.6: *Object list*

	ImageNo	Objects	RelativeArea %	Colonies/ml	SampleVolume	DilutionFactor	DateTime	ImageFile	SettingsProfile
	1	22	1.6649	22000.0000	1.0000	1.0000	9/17/2009 1:35:52 PM	Img___1	white4x

Figure 8.7: *Count list*

8.2 Reference

Once the CellExplorer tab is selected in the component control panel of the *mmi CellTools* main window, CellExplorer becomes the active component. It adds its own menu items group in the main menu and shows its own control panel.

8.2.1 Menu reference

The CellExplorer menus consists of four main-menu groups:

- File
- Edit
- View
- Tools

The main menu “Tools” is not used yet. It is reserved for future use.

8.2.1.1 File Menu

Open Opens the window for opening a saved counting session

Save Quick save of the current counting session, when it has already been saved before

Save as . . . Opens the window for saving a counting session

Printer Setup Opens the window for printer setup

Open Image file Opens the window for loading a photo

8.2.1.2 Edit Menu

Copy Copies the current photo to the clipboard of your PC

Undo Cancels the last change of settings

Reset Resets the current photo to its initial state

Calculate Starts the analysis

8.2.1.3 View Menu

Image View Shows the current photo

Object List Shows the object list

Count List Shows the count list

Panel/Settings Switches the panel between settings and projects (see section 8.2.4)

8.2.1.4 Tools Menu

The main menu “Tools” is not used yet. It is reserved for future use.

8.2.2 Zoom function

The *mmi CellExplorer* contains a comfortable zoom algorithm. Moving the slider on the right hand side of the displayed image zooms the image in or out.

With the **zoom in** function the *mmi CellExplorer* zooms into a specific part of your image. Draw a rectangle over the respective area with the mouse button held down. The mouse pointer will change to a cross shape.

With the **zoom out** function the *mmi CellExplorer* zooms out to the original image size.

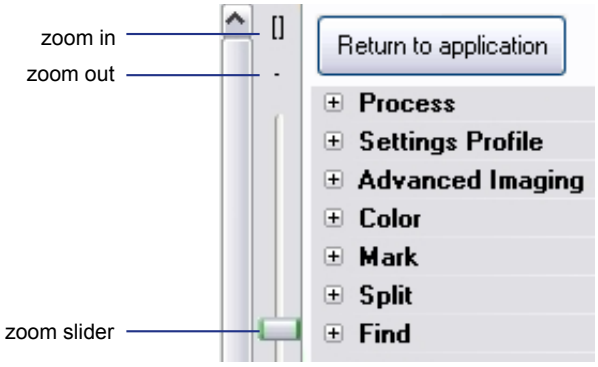


Figure 8.8: *Zoom controls*

8.2.3 Control panel

The *mmi CellExplorer* controls are subdivided into control panels that can be opened and closed on demand by clicking on the button to the left of the panel's title (Fig. 8.9).

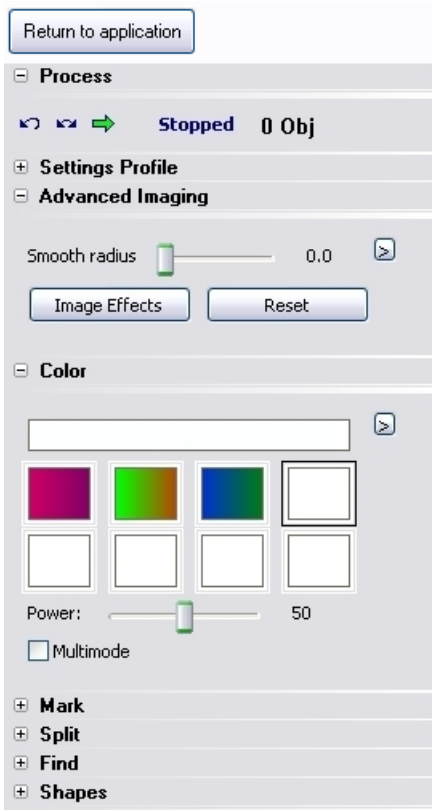


Figure 8.9: *The mmi CellExplorer control panel for cell recognition*

Most panels contain a **preview** button. When clicked, CellExplorer will perform all calculations defined on the respective tool panel on the original image. Use the button to check the settings you have defined so far.

8.2.3.1 Process tool panel

The process panel (Fig. 8.10) contains the following controls (from left to right):

Figure 8.10: *Process panel*

Undo Undo the last change when editing settings

Reset Reset the current photo to its initial state

Calculate Performs a complete analysis on the current image using all parameters defined in the control panel. After this calculation, the objects can be transferred to *mmi CellCut* or *mmi CellEctor*.

Objects Displays the number of counted objects.

8.2.3.2 Settings profile panel

Save your settings by entering a name in the settings profile toolbar and clicking **Save**.

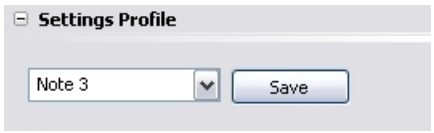


Figure 8.11: *Settings profile panel*

8.2.3.3 Advanced imaging panel

The advanced imaging functionalities allow preprocessing and optimizing the image before an object search is performed.

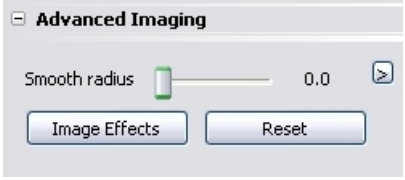


Figure 8.12: *Advanced imaging panel*

Smoothing You can smooth your image with the smoothing function in order to round over edges and achieve a better counting and splitting result. By adjusting the **Smooth radius** slider, you can define the level of smoothing.

The radius used for the calculation of the smoothing can be set with the slider:

- 0 is a small radius
- 10 is a large radius

By clicking the **Check** button, you can verify the effect.

Image Effects For manipulation of digital images, the *mmi CellExplorer* contains additional image editing algorithms. Pressing the **Image Effects** button opens a dialog (Fig. 8.13). You can see two small versions of your image. The left image shows a copy of the original, while the right one directly displays the effects of the changes made by adjusting the controls.

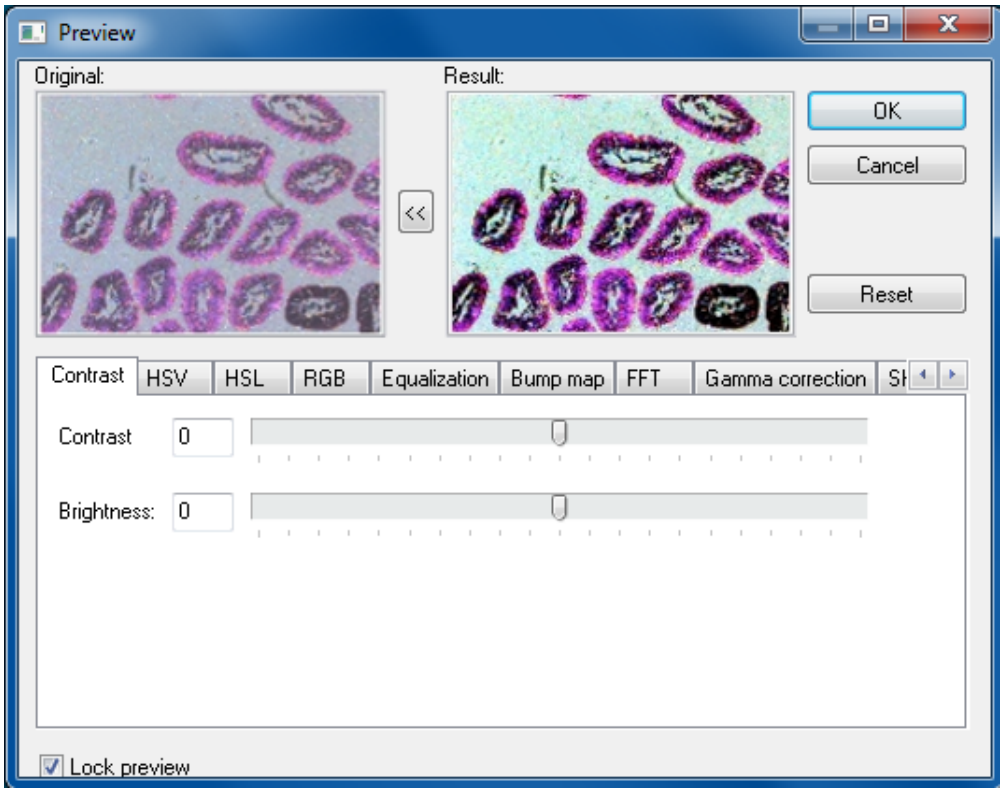


Figure 8.13: *Image effects dialog*

How to use the combination of several image effects:

Evaluate the best set of image effect parameters:

Procedure

1. Open the Image Effect panel
2. Change a parameter and press **Copy Results to Original**
3. Change as many effect parameters and press **Copy Results to Original** until you found the best set of parameters
4. Close

Set the best set of image effect parameters for the use in combination with the green arrow key

Procedure

1. Open the Image Effect Panel
2. Set the best set of parameters without using the **Copy Results to Original** buttons and close the Image Effect Panel with the **OK** button.

Contrast and Brightness Optimize the image contrast and brightness by adjusting the sliders.

The HSV register The HSV register contains further important functions for image editing. It mainly helps with manipulating color images with a color depth of up to 32 bits. The three sliders modify the hue, saturation and intensity/value. New colors are defined with the cursor in the color field located at the lower part of the register. The primary colors are defined with the rainbow tool displayed in the larger part of the window.

The HSL register In the HSL register, you can further adjust the colors, applying the same principles as described for the HSV register.

The RGB register In the RGB register, you can control the individual primary color values. All true colors can be modified by adjusting the three sliders for red, green and blue.

The Equalization register In the Equalization register, halftones can be controlled exactly. The distribution and frequency of the halftones (0–255 for 8-bit images, 0–65535 for 16-bit images) is displayed in the histogram. By adjusting the sliders, halftone ranges can be shielded from the image. Just move the slider with the left mouse button held down. You can also edit color images.

Just click into the small windows below the word “histogram”. The respective primary color will be displayed. Now the individual distribution of the colors red, green and blue (RGB) can be filtered.

Bump Map filter The Bump Map Filter produces a surface structure like those of freeze-cuts. It simulates a metallic surface where objects show high contrasts. You can control the illumination from punctual to flat by adjusting the sliders left/right (Left) and top/down (Top). You can also dye the surface. In order to do so, just move the mouse pointer within the color window.

In case you would like include the original colors of your object into the metallic surface, increase the percentage value below the Source image quantity.

Fast Fourier Transformation The fast fourier transformation searches for edges in the image. This transformation can mainly be used to reduce background noise.

Gamma correction The Gamma correction is a special brightness and contrast Special function. Especially very low light images can be improved by using the gamma correction.

Sharpen With the sharpen function you sharpen the contour of the objects.

8.2.3.4 Color tool panel

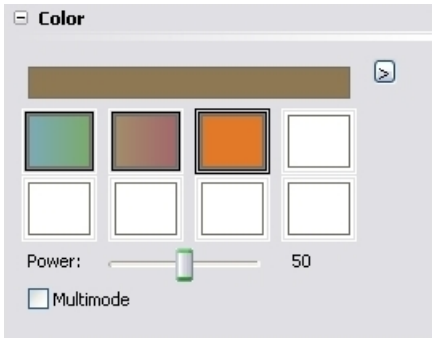


Figure 8.14: *Color panel*

Color fields for different object types Open the color panel to choose up to eight color gradients as settings for counting eight different object types. This enables you to find, count and measure object types with different colors simultaneously; the counting results are shown separately for each color. The color fields are characterized in Fig. 8.15.

(a) Color field inactive for editing and inactive for object searching

(b) Color field active for editing (only one color field can be edited at once), but inactive for object searching

(c) Color field active for object search but inactive for editing.

Figure 8.15: *Color fields state indicators*

Standard color selection With a left mouse click on the displayed image, the color under the cursor will be entered into the left side of the first color field.

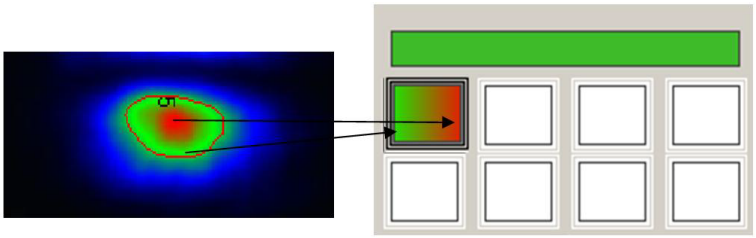


Figure 8.16: *Standard color selection*

A right mouse click on the displayed image will choose the “end-color” of the color gradient to search for.

If the objects are small, it can be tricky to hit the right color. By using the zoom options of the *mmi CellExplorer* as described in section 8.2.2 the selection will be very easy and precise.

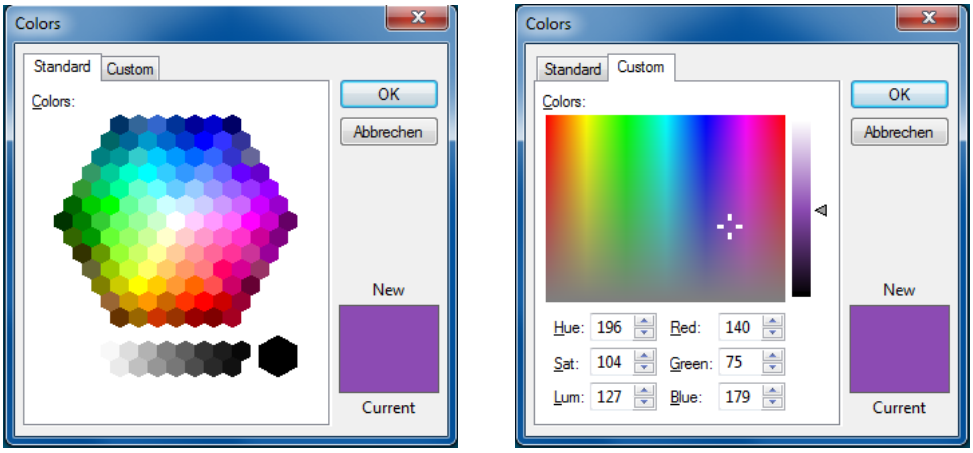
Advanced color selection With a double left mouse click in the right or left part of the color field a standard color menu is opened to select a new colour for the right or left part of the color field. Select the Custom file card in the Color menue to customize the favoured color.

The **Power** slider (Fig. 8.14) adjusts the intensity of the marking. You should try to get homogeneous dark objects and a light background

- 100 is the lowest intensity
- 0 is the strongest intensity

Important: Please try to obtain objects as dark as possible!

Multimode If multimode (Fig. 8.14) is selected the parameters in the lower setting panels defining the object shape, like object area, object roundness. . .



(a) Standard colors (b) Custom colors

Figure 8.17: *Advanced color selection*

will be handled separately for each color set. If multimode is not selected the set of shape parameters will be applied to all color sets.

8.2.3.5 Mark tool panel

After selection of the colors the image will appear as a black and white image. The darker the pixels are the better will the color match the selected color.

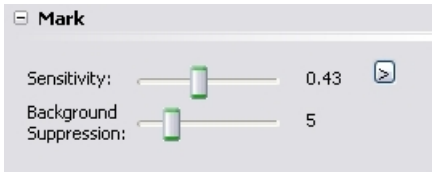


Figure 8.18: *Mark tool panel*

In the **Mark** tool panel (Fig. 8.18) objects can be separated from the surroundings of similar color by adjusting **Sensitivity**:

- 0.01 is the lowest intensity
- 1.00 is the strongest intensity

The **Background suppression** filters the background:

- 1 means low filter suppression
- 20 means high filter suppression

For small objects high filter suppression is recommended, while large objects should be selected with a low filter suppression.

By applying the Mark function the candidate pixels for an object will be labeled red.

> Important: Please try to mark all objects in red with a white background.

8.2.3.6 Split tool panel

The mmi CellExplorer contains a mode for automatic splitting of objects. In microscopic images of cells or structures the relevant objects are often located next to each other in a way that the computer detects those objects as one single structure. The toolbar split is used for separating these overlapping objects:

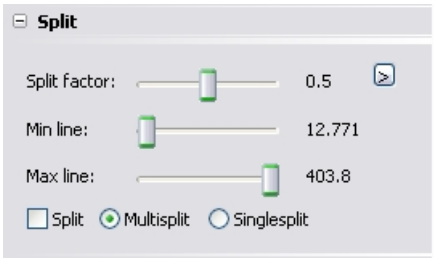


Figure 8.19: *Split tool panel*

For the separation of overlapping objects activate the box **Split**.

Adjusts the intensity of the split factor:

- 0 is a low split factor
- 1 is a high split factor

The **Min line** slider selects the shortest length of the split line. If you select a very small value, many small segments of larger objects are separated as individual objects. If you enter a very large value, overlapping objects are no longer separated.

The **Max line** slider selects the longest length of the split line. The value is preset to maximum. If large individual objects are separated into two parts, you have to decrease the value.

The split feature allows the separation of overlapping objects. The feature **Singlesplit** separates objects once, while the feature **Multisplit** cross-checks after separating whether another separation of the objects is necessary. Separating with **Multisplit** is time-consuming!

8.2.3.7 Find tool panel

With the find tool panel you may adjust the size and roundness of the selected objects.

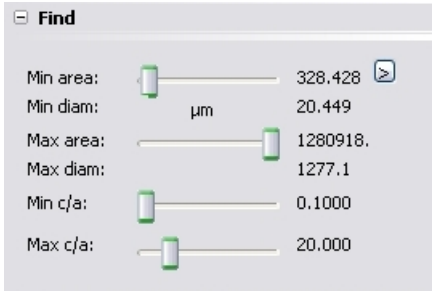


Figure 8.20: *Find tool panel*

Min area and **Min diam** define the smallest object area and diameter, which are counted. **Max area** and **Max diam** defines the largest object size and diameter, which are counted.

Use **Min c/a** and **Max c/a** to filter by roundness: All objects with roundness between the **Min c/a** and **Max c/a** value are counted.

Definition of the roundness in *mmi CellExplorer*:

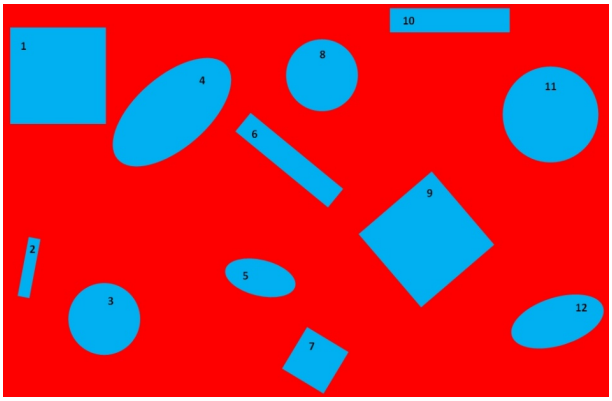
$$\frac{c}{a} = \frac{\text{circumference}^2}{4\pi \cdot \text{total area}} \tag{8.1}$$

The roundness will separate different geometrical shapes such as circles, squares, rectangles and ellipses. The results are independent from shape size and allow you to separate shapes by geometry.

> Attention: In binary images, objects appear as a collection of pixels; therefore, objects are never entirely round or rectangular.

Table 8.1: *Roundness of simple objects*

geometry	roundness
circle	≈ 1.0
square	≈ 1.5
rectangle	≈ 2.0
ellipse, 1:2	≈ 4.0



(a) Original image

	ObjectNo	Unit	Diameter	TotalArea	Circumference	Roundness
	1	µm	1184.9754	702083.3185	3351.6165	1.5708
	2	µm	534.9537	54970.8773	1331.2057	4.0887
	3	µm	632.2779	311343.6148	2088.5129	1.0085
	4	µm	1260.5487	622489.1109	3213.6582	2.0048
	5	µm	630.9560	155816.8586	1611.3605	2.0067
	6	µm	1071.0476	221631.1996	2606.0141	4.0651
	7	µm	600.6331	181413.0935	1819.0820	1.5619
	8	µm	632.4457	311378.9932	2084.1057	1.0089
	9	µm	1192.8757	714720.4644	3475.2633	1.5637
	10	µm	1066.2970	219678.3148	2511.0524	4.0650
	11	µm	841.7502	553172.3134	2779.7572	1.0060
	12	µm	840.5515	276778.9687	2144.4515	2.0049

(b) Results

Figure 8.21: *Example for calculating the roundness of different objects independent from size*

8.2.3.8 Shapes toolbar

With the shape toolbar the contours of the selected objects can be optimized.

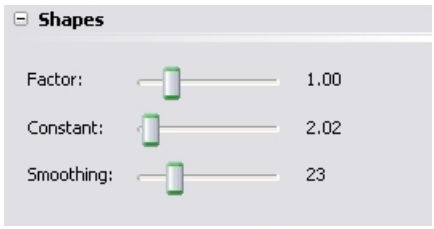


Figure 8.22: *Shapes tool panel*

The **Factor** defines a percentage correction of the shape size:

- Factor < 1.00: reduction of the shape size (Min: 0.8)
- Factor = 1.00: no correction
- Factor > 1.00: increase of the shape size (Max: 1.2)

The **Constant** parameter adds a pixel correction to the shape size:

- Constant = 0.00: no pixel correction
- Constant > 0.00: shape size increases by “constant” pixels (Max: 50)

The pixel correction can be used to compensate for the laser cutting width independent from the object size.

With the **Smoothing** feature the contours of the selected objects can be smoothed:

- Smoothing = 0: no smoothing
- Smoothing > 0: increase smoothing of object contours (Max: 100)

8.2.4 Project panel

The *mmi CellExplorer* project panel shows all images contained in the current session. You can choose whether the image is displayed in the work image panel using the checkboxes on the left of the images. You can also delete an image from the current session by marking it with the mouse and clicking the **Delete** button.

A Service

Service should only be performed by qualified MMI personnel or our designated representative(s). The MMI system contains no user-serviceable parts.

For questions about your instrument (technical, consumables, warranties) please contact:

Europe and Asia:
✉ Molecular Machines & Industries AG (MMI)
Flughofstrasse 37
8152 Glattbrugg
Switzerland
☎ +41 – (0)44 – 8 09 10 10
FAX +41 – (0)44 – 8 09 10 11

North America:
✉ MMI Molecular Machines & Industries Inc.
P.O. Box 348
Haslett, MI 48840
USA
☎ +1 – (603) 629 9536
FAX +1 – (321) 978 0304

www.molecular–machines.com

service@molecular–machines.com

B List of Keyboard Shortcuts

General

Caps Lock	Suppress continous stage movement	20
Ctrl + P	Stage movement settings	20
Esc	Interrupt current process	23, 83
F1	Display user manual	26
Space	Switch between moving and drawing mode	19

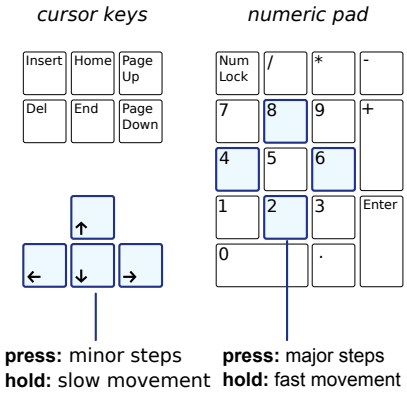
Camera control

Ctrl + R	Open camera settings	14
Ctrl + S	Save an image	16
Ctrl + W	Set white balance	15

Microscope control

Ctrl + 1	Define first sample plane point	98
Ctrl + 2	Define second sample plane point	98
Ctrl + 3	Define third sample plane point	99
F6	Open/close fluorescence shutter	102
F7	Switch between camera and binocular	96
F8	Define z drive offset for current slide	100

Navigation


cursor keys
numeric pad
Insert
Home
Page Up
Del
End
Page Down
Num Lock
/
*
-
7
8
9
+
4
5
6
1
2
3
Enter
0
.
press: minor steps
hold: slow movement
press: major steps
hold: fast movement

C Technical data

C.1 Required minimum workspace

The table top for the microscope, laser, optical equipment, computer monitor and keyboard requires a minimum workspace of 1.20 m $\times$ 0.90 m.

The computer should be positioned under or near the table. The camera–computer connection cable is 2 m long to ensure reliable data transfer.

C.2 System components

C.2.1 Microscope

Inverted or upright research microscope with fluorescence port.

Supported microscope models:

- Olympus IX-71 (non-motorized)
- Olympus IX-81
- Nikon Ti (S, U, E)
- Nikon Ni (U, E)
- Nikon TE 2000 (S, U, E)

C.2.2 Stage

Scanning stage with stepper motors.

Scanning area:	120 $\times$ 100 mm^2
Repositioning accuracy:	< 1 µm
Step resolution:	0.078 µm

C.2.3 Digital camera

mmi CellCamera MXF285c

- IEEE1394b (FireWire) color CCD camera
- 2/3" interline transfer frame readout CCD
- Temperature controlled active Peltier cooling
- signal to noise ratio: > 62 dB
- On board integrated color processor for high quality color calculation
- 1392 $\times$ 1040 pixels with up to 20fps
- Ultra high sensitivity

mmi CellCamera DXA285cF

- IEEE1394a (FireWire) interline transfer color CCD camera
- 2/3" interline transfer frame readout CCD
- Super HAD technology
- signal to noise ratio: > 56 dB
- 1392 $\times$ 1040 pixels with up to 15 fps
- Ultra high sensitivity

mmi CellCamera DXA285F

- IEEE1394a (FireWire) interline transfer CCD camera
- 2/3" interline transfer frame readout CCD
- Super HAD technology
- 1392 $\times$ 1040 pixels with up to 15 fps
- Ultra high sensitivity

Andor iXonEM+ EMCCD Camera 897

- EMCCD Technology: Even single photon signals are amplified above the noise floor. Full QE of CCD chip is harnessed (no intensifier).
- RealGain^{TM}: Absolute EMCCD gain selectable directly from a linear and quantitative scale.
- TE Cooling to −100 ℃: Critical for elimination of dark current detection limit.

C.2.4 Computer

Suitable computer workstation models are preselected and tested by MMI. Only use computer hardware supplied through MMI. Before performing hard-

ware modifications, contact service.

mmi CellTools supports Microsoft Windows XP, Windows 7 and Windows 8 in 32-bit and 64-bit configuration.

C.2.5 3D Manipulator

Travel range:	25 mm
Resolution:	0.025 µm

C.2.6 *mmi CellPump*